TECHNIQUES FOR THE ANALYSIS OF ORGANIC MICRO-CONTAMINANTS AND THEIR APPLICATION TO ENVIRONMENTAL MONITORING.

(C) DAVID JOHN PIRIE, 1996

Thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy
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Collaborating Establishment: Clyde River Purification Board .

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David John Pirie

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Summary

The rapid increase in the commercial use of organic chemicals since the mid twentieth century has led to a need for governments to monitor and regulate the levels of these chemicals introduced into the environment. This study was carried out to improve and expand the existing analytical methodology used in environmental organic contaminant monitoring, and to apply these methods to field investigations of two sites (Annick Water and Irvine Bay) where organic chemicals were suspected of having an adverse effect on the environment.

Analytical organic contaminant monitoring methods were reviewed and the necessary component chemical and instrumental techniques required for the determination of both organochlorine and pyrethroid contaminants in an analytical method identified and investigated.

A robust modular analytical method was developed, optimised and validated for river water, effluent, sediment and biota matrices. The optimised methods consisted of:

1. Duplicate liquid-liquid extractions of a 1 litre aqueous samples with 50mls of hexane,
2. Solid phase extractions of 1 litre aqueous samples using octadecyl bonded silica adsorbents,
3. Soxhlet extraction of solid matrices for 7 hours using MTBE as the extracting solvent,
4. Removal of coextracted sulphur using tert butyl ammonium sulphate or copper powder,
5. Cleanup of extracts using normal phase adsorption chromatography with either florisil, alumina or silver nitrate impregnated alumina as the adsorbent,
6. Separation of analyte classes using silica gel adsorption chromatography,
7. Separation and detection of the analytes by gas chromatography using a 60m medium polarity capillary column and electron capture detection.

The suitability of the methods to field investigations and regulatory use was demonstrated by determining the fate and effects of a range of organic contaminants released by the textile and wool processing industry on a freshwater (Annick Water) and a marine (Irvine Bay) ecosystem.

Permethrin, isomers of HCH, dieldrin, DDT and metabolites of DDT were detected in the effluent from Stewarton STW which discharges into the Annick Water. Permethrin and isomers of HCH were the compounds detected most frequently and at the highest concentrations. The concentrations of permethrin detected were likely to cause a breach of the freshwater environmental quality standard in the Annick Water immediately downstream of the STW.

Permethrin, dieldrin, HCH isomers, DDT, metabolites of DDT and PCBs were detected in sediments from the Annick Water. Comparison of the concentrations of contaminants detected in the sediments with published sediment contaminant concentrations, derived sediment quality standards, and published sediment toxicity data indicated that permethrin was the most significant contaminant detected in the sediments.

Comparison with invertebrate biotic index scores for the Annick water and fish population studies of the Annick water, indicated that the sediments between the STW and Chapeltoun were acutely toxic to invertebrates.

Invertebrate biotic index scores from the lower reaches of the Annick Water were lower than could be explained by comparison with published sediment toxicity data. Faster degradation of trans permethrin in the environment to form a more toxic mixture of permethrin isomers, synergistic activity with organophosphorus pesticides and the negative insecticidal temperature coefficient of permethrin were suggested as a possible explanations of these low biotic index scores.

Permethrin was not detected in eels caught from the Annick water or the Glazert Burn, suggesting that eels can metabolise permethrin. Dieldrin was the major contaminant detected in eels downstream of the STW. The concentrations of dieldrin detected in these eels were so high as to suggest that regular human consumption of these eels presented a significant hazard to human health and the viability of wild populations of fish-eating birds and aquatic mammals.

Similar organochlorine contaminants were detected in effluents from Irvine and Garnock Valley sewers and in sediment from Irvine Bay. Contaminants found in the Irvine Bay sea outfall effluents include permethrin, HCH isomers, dieldrin, DDT and metabolites of DDT. Permethrin and isomers of HCH were the compounds most frequently detected and at the highest concentrations.

The concentration of permethrin detected in the effluent from the valley sewers in typical conditions were likely to cause a slight breach of the EQS at the edge of the mixing zone. In adverse conditions the concentrations of permethrin in the effluent from both valley sewers were likely to result in permethrin concentrations at the edge of the mixing zone approximately 5 and 10 times higher than those permitted by the EQS.

Permethrin, dieldrin, HCH isomers, DDT, DDT metabolites and PCBs were detected in sediments from Irvine Bay. Permethrin and G HCH were the major contaminants detected. The spatial distribution of organic carbon normalised contaminants indicated that the valley sewers are the major sources of these contaminants within Irvine Bay. Slightly elevated concentrations of dieldrin, DDT and metabolites of DDT were also observed at sites close to the valley sewers indicating that the valley sewers are minor sources of these compounds. PCBs were detected at low concentrations at all sites from within Irvine Bay. No significant elevation in PCB concentrations was observed at sites close to the valley sewers, indicating that the valley sewers are not significant sources of PCBs.

The concentrations of PCBs, dieldrin, DDT and metabolites of DDT detected in the sediments were unlikely to have adverse biological effects. The concentrations of permethrin detected in sediments from within the mixing zone were similar to those shown in laboratory toxicity tests to have a toxic effect on freshwater organisms, the concentrations of permethrin detected in sediments outwith the mixing zone were unlikely to have adverse effects on the benthic fauna of Irvine Bay.

Comparison with biotic indices indicated that biological impact followed a similar pattern to the organochlorine contaminant levels. It was not possible to conclude if the biological impact was related to a specific contaminant.

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ABBREVIATIONS

µm	micro metre
µg/l	micrograms per litre
APHA	American Public Health Association
ASPT	Average Score per Taxon
ASTM	American Standard Testing Methods
BMWP	Biological Monitoring Working Party
BOD	Biochemical Oxygen Demand
C18	Octadecyl
C8	Octyl
CRPB	Clyde River Purification Board
CUMEC	cubic metre per second
DC	Direct Current
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethane
DOE	Department of Environment
EC	European Community
ECD	Electron Capture Detector
EI	Electron Impact
EP	Equilibrium Partitioning
EQS	Environmental Quality Standard
EU	European Union
FDEP	Florida Department of Environmental Protection
FID	Flame Ionisation Detector
GC	Gas Chromatograph
GC/MS	Gas Chromatography Mass Spectrometry
GPC	Gel Permeation Chromatography
GVS	Garnock Valley Sewer
HCB	Hexachlorobenzene
HCBD	Hexachlorobutadiene
HCH	Hexachlorohexane
HPLC	High Pressure Liquid Chromatography
ICES	International Council For The Exploration of the Seas
IPA	iso propyl alcohol
IVS	Irvine Valley Sewer

JMG	Joint Monitiring Group
JMP	Joint Monitoring Program
Km	Kilometre
LC	Liquid Chromatograph
LV	Limit Value
m	metre
mins	minutes
mm	millimetre
MR	Mean Response
MS	Mass Spectrometry
MTBE	Methyl tert butyl ether
NBS	National Bureau of Standards
NCI	Negative Chemical Ionisation
ND	not detected
ng/g	nanograms per gram
ng/l	nanograms per litre
NRA	National Rivers Authority
NSTF	North Sea Task Force
°C	Degrees Centigrade
OCPs	Organochlorine Pesticides
PCBs	Polychlorinated Biphenyls
PCI	Positive Chemical Ionisation
PEL	Probable Effects Level
ppm	Parts Per Million
RF	Radio Frequency
RIVPACs	River Invertebrate Prediction and Classification System
RSD	Relative Standard Deviation
SEC	Size Exclusion Chromatography
SEPA	Scottish Environment Protection Agency
SFE	Super Critical Fluid Extraction
SIM	Selected Ion Monitoring
SIR	Selected Ion Recording
SOAEFD	Scottish Office Agriculture, Environment and Food Department
SOEnD	Scottish Office Environment Department
SPE	Solid Phase Extraction
SQAGS	Sediment Quality Assessment Guidelines
STW	Sewage Treatment Works
TEL	Threshold effects level
TOC	Total Organic Carbon
UK	United Kingdom

UKSCA	United Kingdom Standing Committee of Analysts
US	United States
US EPA	United States Environment Protection Agency
WEA	Weight of Evidence Approach

Chapter 1
Introduction

1.1 General

Throughout history man has been creating new chemicals and it has been estimated that during the whole period of human development, around six million chemical compounds have been synthesised (Alloway & Ayres, 1993). The production and use of these new chemicals has increased dramatically in the past fifty years. At present approximately one thousand new chemicals are synthesised each year and between sixty and ninety five thousand chemicals are in current commercial use (Alloway & Ayres,1993). A large number of these chemicals are essential to human welfare helping to control and eliminate agents that effect the spread of pests and disease, thus helping to maximise the production of food and fibres which sustain the world's growing population and maintain our modern way of life. In agriculture alone it is estimated that the world wide annual use of insecticides is between sixteen and twenty four thousand million tonnes (Greve & Smith, 1992).

Insects are the most numerous of living organisms and the nearly one million described species constitute approximately 70% of all animal species (Cramer, 1967). Of these, about 1% are considered significant pests, attacking humans, domestic animals, food supplies and fibres. Estimates suggest that world wide agricultural losses due to insects are about 14% of production.

Since food and textile production began, man has attempted to lessen these losses by the use of chemicals. The earliest pesticides were mainly inorganic chemicals such as sulphur and arsenic compounds. These compounds had limited success often presenting as great a hazard to humans as to the pest species themselves. The synthesis of dichlorodiphenyltrichloroethane (DDT) and the discovery of its contact insecticidal properties in the early forties began an era of chemical pest control which has seen the synthesis and evaluation of thousands of synthetic organic chemicals as insecticides.

Following the tremendous success of DDT, a number of different types of organochlorine compounds such as the hexachlorocyclohexanes and cyclodienes were also found to be potent contact insecticides. Collectively these and other compounds are known as organochlorine insecticides. This diverse group of artificially produced chemicals are characterised by a cyclic structure and a variable number of chlorine atoms. These chemicals came to prominence during the rapid growth of the petrochemical and chlorine industries in the late forties and early fifties. By the late fifties organochlorine compounds were the most widely used insecticides in the world (Cremlyn, 1991).

The benefits to mankind from the use of these chemicals has been enormous. They played a large part in the post second World War farming/food production revolution which allowed agricultural production to increase dramatically. In addition to benefiting mankind through increased crop production, public health applications of organochlorine insecticides and DDT in particular has saved the lives of millions of people. For example, it has been estimated that between 1942 and 1952 the use of DDT in the World Health Organisations global malaria elimination program saved five million lives and prevented more than one hundred million illnesses (Metcalf *et al.*, 1962).

However, the widespread and indiscriminate use of organochlorine insecticides resulted in an increasing recognition of not only practicable difficulties in their use but also a gradual awareness of adverse effects on non-target organisms. Difficulties in use included increased resistance of successive generations of insects resulting in resurgence of pests, outbreaks of secondary pests due to elimination of natural enemies and adverse human health effects. Public awareness was greatly influenced by the publication of the book 'Silent Spring' by Rachel Carson in the mid sixties. This book focused attention on links between cancer and insidious pesticide pollution, forecasting the elimination of the bird population due to effects on egg survival.

Following publication of this book there was a significant increase in environmental monitoring and research to gather evidence of possible contamination of terrestrial and freshwater species by pesticides such as DDT. Gas liquid chromatography became available for environmental analysis during this period and began to be used for the identification of individual chlorinated compounds. Using this technique, analytical chemists measuring DDT in bird tissue encountered groups of unknown peaks. These peaks were identified in 1966 by Jensen (Jensen, 1966) as polychlorinated biphenyls (PCBs). As a result of this identification environmental concern was additionally focused on this and other groups of synthetic organic chemicals such as hexachlorobenzene and hexachlorobutadiene which were increasing in industrial use.

Jensen's historical analytical work in identifying PCBs in environmental samples highlighted the problems of analysing complex matrices for organochlorine residues and showed that earlier failures to properly resolve contaminants must have contributed to an over-estimation of DDT and the metabolite dichlorodiphenyldichloroethene (DDE) in the environment.

During the past thirty years world-wide surveys have established the global impact of organochlorine pollution. Organochlorine compounds have been shown to accumulate and persist in the environment and are now considered ubiquitous global contaminants (Bommanna *et al.*, 1994). However, the hazards posed by these chemicals are still not fully understood. In this period surveys have linked them to dramatic effects on wildlife populations such as reductions in the otter population in the UK during the late fifties, the reduction of the farmed mink population around the great lakes in the US during the early sixties, the reduction of sea bird and bird of prey populations in the seventies, reproductive failure of alligators in Florida in the late eighties, and more recently,

allegations of increased vulnerability of seals in the North Sea and dolphins in the Mediterranean to infections such as distemper (Colborn *et al.*, 1996).

As a result of these reported environmental effects the production and use of most organochlorine compounds has been terminated or severely restricted in the majority of developed countries. Alternatives have been found for PCBs in the electrical industry and organophosphorus and synthetic pyrethroids have replaced organochlorines as the dominant insecticides in developed countries. Unfortunately, due to their low cost organochlorine compounds such as lindane, DDT and dieldrin are still widely used in developing countries. In addition these compounds are still present in materials and equipment currently in use but produced before restrictions on the use of organochlorine compounds were in place. For example it has been estimated that of the PCBs produced throughout the world 57% are still in use, 27% are in landfills and 16% are in the environment (Klamer *et al.*, 1991). These compounds are still finding their way into the environment and therefore present a significant long term risk to human health and to the environment.

To minimise the risks from these chemicals governments and regulatory bodies have responded by taking action, introducing limits and controls on the emission of pollutants into the environment. In addition to controlling the use of compounds such as organochlorines that have been shown to have significant adverse effects, governments need to gather data on the distribution and long term effects of these compounds. In order to control and eliminate risks from chemicals it is necessary to determine the quantities of these chemicals both in the environment and in discharges to the environment.

Analysts who are required to monitor for these compounds need, therefore, to develop techniques to separate and quantify a growing number of contaminants. The contaminants of the sixties and seventies have not gone away but are continuing to appear as they are mobilised into the environment while at the same time new chemicals continue to be introduced. Methods must be validated to ensure that new compounds are not mis-identified. Against this background of more complex residues, analysts are facing severe financial restrictions on analytical resources. Methods should be developed to maximise returns in terms of contaminants quantified per unit of analytical effort.

The theme of maximising the amount of information gained by studying wider ranges of chemical mixtures was highlighted at the Intermediate Ministerial Meeting on the North Sea held in Copenhagen in 1993. Conclusions from this indicated that there was a lack of high quality data on existing dangerous substances such as PCBs and DDT and even less data on new compounds such as permethrin (Anon., 1993 a). This lack of data is confirmed in the most recent North Sea Quality Status Report (Anon., 1994a) which was unable to quantify inputs to the North Sea of even well studied compounds such as PCBs due to the lack of good quality data. Attempts to address this lack of data has highlighted the need for analytical techniques that are capable of detecting large numbers of hazardous substances in a single analytical method. This lack of suitable analytical methods has been recognised by the National Rivers Authority (NRA) (Environment Agency from 1st April 1996), and they have recently recommended that analytical techniques, particularly for pyrethroids and fungicides, be developed to allow their determination alongside existing analytical analytes (NRA 1995).

The control of discharges from wool processing and textile industries are specific examples of areas where improvements in analytical methods could have significant benefits. Effluent from wool processing and textile industries contain a wide variety of synthetic organic chemicals, for example sheep dip compounds, mothproofing chemicals, dyes and detergents. NRA recommendations (NRA 1995) highlighted the wool processing and textile industries as significant point sources of synthetic organic contaminants that require strict regulation. As the number of compounds and the level of regulation increase, the financial costs of monitoring increase exponentially (Alloway & Ayres, 1993). To ensure environmental control does not impose unacceptable burdens on both industrial producers and regulators it is essential that accurate, reliable and robust analytical methods which maximise the amount of information gained are used. At present halogenated micro-contaminants are determined using separate analytical techniques optimised for a specific class of contaminants e.g. PCBs, organochlorine pesticides, or synthetic pyrethroids. This results in samples having to be repeatedly analysed using a number of analytical methods. An analytical method capable of determining all of these classes of analytes in one analysis would greatly simplify and potentially reduce the costs of monitoring these industrial processes.

Within Scotland the principle legislation used to regulate the aquatic environment is the Control of Pollution act 1974. This allows River Purification Authorities (RPAs) (Scottish Environment Protection Agency since 1st April 1996) to control discharges to the aquatic environment by a system of consents. The RPAs use these consent granting powers to ensure, wherever possible, that water quality meets standards set in a number of EC Directives. The Directive which relates to synthetic organic chemicals is Directive (76/464/EEC) and is commonly referred to as the dangerous substance Directive. This Directive does not in itself control the discharge of specific substances but classifies substances into two categories, List I (pollution by substances to be eliminated) and List II (pollution by substances to be reduced). In broad terms List I substances are

considered to be most harmful and are controlled by environmental quality standards set at EC level. List II substances are considered less harmful and are controlled by environmental standards at national level. Consultants for the EC have selected one hundred and twenty nine substances as potential List I substances (known as the 129 priority list). However, at present only eighteen of these have been formally classified as List 1 substances by the EC in 'daughter' Directives such as 84/491/EEC.

Environmental quality standards (EQSs) are set in these Directives and are transcribed into UK legislation (Statutory Instruments No. 2286,1989., and SOEnD Circular 6/1993.). Directive 76/464 leaves the identification of List II substances and the setting of appropriate EQSs to individual member states. In the UK, DoE has set standards for a number of substances via Circular 7/89, a form of government advice note. The List 1 and List II compounds are listed in Appendix 1.

In addition to the requirements of the Directives, UK Ministers have agreed to at least halve the input loads of thirty six priority hazardous substances discharged to the North Sea by 1995. These substances and their associated target reductions are listed in Appendix 2. Further common actions agreed at the 3rd North Sea Conference were the identification of substances whose use should be strictly controlled or banned. These substances are listed in Appendix 3.

From the above legislation classes of compounds which were: (i) chemically similar, (ii) likely to be associated with industrial mothproofing, and (iii) in need of regulation, were identified and selected for study. The selected classes of compounds were as follows: halogenated synthetic pyrethroids, hexachlorohexanes (HCH), DDT, Cyclodienes and "Drins" (e.g. dieldrin and endrin), hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), and PCBs. Brief outlines of the compounds contained in each class, their properties and uses are given in Appendix 4.

There is a clear need for robust analytical methods which can determine these compounds in a range of sample matrices using similar analysis schemes.

1.2 Scope and Summary

The work reported in this thesis covers three areas: (i) analytical method development, (ii) analytical method validation, and (iii) the application of these analytical methods to environmental research. The specific aims of this study were therefore as follows:

(1) To develop a robust analytical method capable of detecting and quantifying a wide range organochlorine contaminants together with halogenated synthetic pyrethroids at environmental concentrations in a variety of matrices.

(2) To estimate the precision and bias of the above analytical methods.

(3) To use the developed analytical techniques to determine the distribution and environmental significance of the discharge of these chemicals from wool processing and textile industries on the Annick Water (Freshwater) and Irvine Bay (Marine) ecosystems.

1.2.1 Method Development

The aim of this work was to develop a method suitable for use in regulating a range of chemicals which were of environmental concern and discharged from wool processing textile industries.

As indicated earlier, a range of analytical techniques have been used for the determination of individual classes of these compounds. As a first step in selecting the techniques most suitable for use in a multi-residue method which would be capable of detecting a range of analytes, a comprehensive review of the existing analytical techniques was carried out.

This review indicated that gas chromatography is the analytical technique best suited for the determination of organic pollutants in water (Hennion & Scribe, 1993). The use of gas chromatography with electron capture and mass spectrometric detection for the determination of these compounds was fully investigated and optimised.

Sample pre-treatment is still regarded as the weakest link and is the time-determining step in these analytical procedures, accounting for about two thirds of the total analysis time. It is also the primary source of errors and discrepancies between laboratories (Majors, 1991). In the environmental literature, most of the sample preparations described are based on manual time-consuming procedures that have been used for decades. There is a real need for developing sample-handling strategies which are more rapid, more reliable (which means that the number of intermediate steps such as transfers, evaporation and derivatisation is diminished) and easily capable of automation (Hennion & Scribe, 1993). A variety of sample preparation strategies were thoroughly investigated and optimised in order to develop modular analytical strategies that met the above requirements and could be applied to a variety of environmental matrices.

1.2.2 Validation

Environmental management depends on accurate information on a wide range of compounds. Unreliable or inaccurate information may lead to large economic losses, for example, large capital works and effluent treatment measures which may be undertaken needlessly, or to unacceptable risks for the environment or human health if appropriate measures are not taken. It is essential that the quality of information on the environment is adequate. Extensive method performance tests were undertaken to fully evaluate the precision of the analytical procedures. The bias of the analytical procedures were estimated by participation in national and international interlaboratory studies. In addition, for compounds where no recognised interlaboratory studies were available, the methods were compared as part of a Community Bureau of Reference (BCR) study to validate a novel lyophilised reference material.

1.2.3 Environmental Monitoring

The Annick Water is a largely rural river in northern Ayrshire. The water of this small river is of the highest chemical class suggesting that the river should support a rich diversity of aquatic life. However, surveys of aquatic invertebrates in the Annick Water downstream of Stewarton have indicated that the aquatic life of the Annick Water is impoverished. The town of Stewarton supports a woollen mill which discharges it's effluent via a sewage treatment works to the Annick Water. Mothproofing chemicals in this effluent have been linked with the poor biological classification of the Annick Water downstream of Stewarton.

Irvine Bay on the eastern edge of the Firth of Clyde is an important fishery and the major nursery for flatfish within the Firth Of Clyde. Irvine Bay also receives large amounts of effluent from textile and wool processing industries and concerns have been expressed over the significance of the discharge of mothproofing chemicals to this environment.

In two separate monitoring programs, the analytical procedures developed in this study were used to determine the concentrations of organochlorine pesticides, synthetic pyrethroids and polychlorinated biphenyls in waters, effluents, sediments and eel tissue from the above locations. The environmental significance of these concentrations were assessed by comparison with concentrations reported by other studies, comparison with environmental quality standards and comparison with biological data. In addition the use of sediment quality standards derived by the equilibrium partitioning approach was investigated.

Chapter 2

Review of Current Analytical Techniques

2.1 General

A prerequisite for decision making in environmental protection and pollution control is the ability to identify and measure xenobiotic materials in ecosystems. Every phase of environmental protection depends upon analytical data. It is not sufficient to merely generate data, this data must be reliable and truly represent the situation. When there is no information on the quality of the data, the decisions based upon them are, at best, questionable.

The aim of this study is to develop a modular multi-residue method capable of determining a wide range of organochlorine and synthetic pyrethroid pesticides in water, biota and sediment matrices. The modules to consist of individual steps for extraction, clean-up, separation and determination of the analytes. The modular nature of the method will allow variations in individual modules to be optimised for particular sample matrices .

Pesticide analysis is broadly divided into two types; a) the identification and quantification of pesticides in formulations, and b) the analysis of pesticide residues. The first is macro analysis determining the percentage active ingredients in formulations and relying on relatively crude analysis methods using spectrophotometric, colorimetric, and chromatographic techniques. The second type are complex analytical separation schemes based on chromatographic techniques. They involve not only the identification and quantification of the parent compound but also the analysis of metabolites, and degradation products.

It is currently possible to detect individual pesticides in the environment at concentrations below 1 ng/g. These detection limits are being pushed ever lower as techniques improve and as governments introduce requirements to demonstrate correlations with sub lethal biological effects data.

Residue analysis has taken two main approaches towards the generation of analytical data.

(1) Multi-residue schemes.

(2) Specific target compound analysis.

Multi-residue methods are for related compound classes, thus we have multi-residue methods for organochlorine pesticides (OCPs), organophosphorus pesticides and carbamate classes. Highly specific target compound analysis have developed for compounds which are not of a broad class or whose individual physical properties (polarity, thermal stability) preclude them from a multi-residue method, e.g. glyphosphate, diquat and paraquat.

Multi-residue methods are of necessity a compromise using the common properties of the class of compounds to be determined. The class members may, however, have subtly different physical and chemical properties leading to a compromise in extraction solvents and clean up methods. The cost benefit in gaining maximum information in a single analysis from valuable samples, has led to the widespread adoption of multi-residue schemes.

Multi residue analysis may be broken down into a series of simple sequential steps:

(1) Sampling, Sample Handling.
(2) Sample Preservation and Storage.
(3) Sample Preparation.
(4) Sample Extraction.
(5) Extract Clean-Up.
(6) Determination.

Procedural steps are discussed in reverse order because features in some prior steps are controlled by limitations of the subsequent steps for example the use of an electron capture detector would preclude the use of electron capturing solvents such as dichloromethane.

2.2 Determination

Environmental sample extracts are complex mixtures. Irrespective of how selective and efficient the methods of extraction and clean up no single detector can determine all of the compounds of interest together. A method of separation immediately before presentation to the detector is therefore necessary. Chromatography is the usual method of mixture separation.

There are two main choices of chromatographic separation for this prior separation, liquid chromatography (LC) and gas chromatography (GC).

Gas chromatography has become the method of choice for both the qualitative and quantitative determination of individual organic compounds in the aquatic environment (Keith & Minear, 1984). Although the method is limited to compounds that can be volatilised either directly or after derivatisation it allows the analysis of many important classes of organic pollutants and natural products, e.g. hydrocarbons, halogenated hydrocarbons, pesticides, phenols and fatty acids.

During the last ten years capillary gas chromatography, in conjunction with selective detectors, has become the most common technique (Barcelo & Lawerence, 1992) for the determination of environmental pesticide residues. Low detection limits, high selectivity, and the relatively low cost of this technique have seen it dominate over packed column GC and liquid chromatographic techniques. The last 5 years have seen an increase in the use of LC techniques particularly for the determination of thermally labile compounds. This study is primarily concerned with organochlorine and mothproofing chemicals as these compounds are suitable for GC analysis. This study has confined itself to capillary GC.

When applying capillary gas chromatography to complex mixtures, the analyst has a large number of control parameters to balance and optimise. These parameters include injection method, column size, choice of stationary phase, carrier gas, and detection method. These options and their effect are discussed in detail in the following sections.

2.2.1 Injection Techniques

The function of an injection system is to transfer the sample quantitatively onto the chromatographic column as a concentrated narrow band. In modern capillary gas chromatography each manufacturer has a variety of individual methods of sample injection. These are all variations on three basic sample injection designs: Split injection, Splitless injection and On-column injection.

2.2.1.1 Split Injection

Split injection was for many years the only means of sample introduction for capillary chromatography. In this system the sample is injected into a heated inlet resulting in rapid volatilisation. A relatively high flow of carrier is passed into the injection chamber forming a

homogeneous mixture of carrier gas and sample. The homogeneous mixture is split into two unequal flows, the smaller portion entering the column while the larger portion is vented. This technique relies on the rapid volatilisation of the sample and works well for the analysis of analytes within a narrow range of boiling points. Samples containing a wide range of components with very different boiling points or thermally labile compounds result in problems in compound discrimination. This occurs because not all of the components are adequately volatilised and this leads to the formation of a non homogenous mixture. This results in lower responses for high molecular weight non-volatile compounds. Because the majority of the sample is vented the technique has poor detection limits.

When used in multi-residue methods for the analysis of trace levels of PCBs and OCPs in environmental samples, split injection gives low response and poor discrimination. For these reasons split injection is considered unsuitable for a multi-residue method.

2.2.1.2 Splitless Injection

Splitless injection is similar to split injection. Prior to injection the instrument configuration is as per split injection, i.e. carrier gas flow enters into the top of a heated inlet where it flows down the insert, at the bottom of the insert it divides into two unequal portions the smaller of which enters the column with the larger portion vented. Just before injection a solenoid valve is actuated to prevent carrier gas being vented. Flow through the injector now passes only onto the column. The sample plus solvent are injected and once volatilised, pass completely onto the column. Sample residence time in the injection chamber is longer than with split injection and therefore lower temperatures may be used to obtain complete volatlisation of the sample. The column may be held at a temperature 10 - 20°C below the boiling point of the solvent resulting in the sample components being trapped in a narrow band at the head of the column. At a predetermined time after injection, the solenoid valve returns to its original state, restoring flow of the carrier to the vent sweeping away any residual sample or solvent to vent.

This technique is most suitable for trace analysis as a larger proportion of the injected sample is placed on the separating column. In addition, the lower volatilisation temperatures are more suitable for thermally labile compounds. Care must be taken to optimise both the split time, injection chamber volume and solenoid actuation time to ensure that the sample is completely volatilised and quantitatively transferred to the head of the column. Poorly optimised splitless injection results in molecular weight/boiling point discrimination and/or poor peak shape. A major disadvantage of this technique is the necessity to use an injection liner. These liners are usually glass and come in a variety of design some with packing such as glass wool and silica. After many injections, particularly of environmental samples, the liners can become coated with high boiling point waxes and lipids which can act as a stationary phase trapping sample components or as sites for sample degradation. To obtain good quality chromatograms these liners need to be frequently changed.

Splitless injection and variations on splitless injection are the most commonly used injection techniques in environmental trace organic analysis.

2.2.1.3 On-column Injection

On-column injection introduces the sample directly into the inlet of the capillary column usually via a retention gap. A retention gap is a short piece (typically 1m) of fused silica capillary tubing without a stationary phase. The column and retention gap are held at temperatures at or below the boiling point of the solvent. The internal volume of the retention gap is not favourable with respect to the expansion in volume that occurs during solvent vaporisation and, therefore, care must be taken to avoid a high inlet temperature which would cause an explosive vaporisation of the solvent with consequent back ejection of a portion of the solvent into the carrier gas lines. The flow of carrier gas through the retention gap slowly removes the solvent from the retention gap. Once the solvent is removed the sample components can be transferred to the head of the column, still at a relatively low temperature, by heating the column inlet area of the retention gap ballistically. As the analytical column is still at a relatively low temperature the sample components are trapped in a narrow band for the subsequent chromatographic analysis.

On-column analysis is an excellent technique for the quantitative analysis of very dilute samples containing analytes of a relatively wide range of volatilities. It is the most controlled injection technique and the most suited for the analysis of thermally labile compounds. All of the sample is injected directly onto the column and therefore it is also the most suited for the analysis of non-volatile compounds. A significant disadvantage of this technique is that since all of the sample is placed onto the column or retention gap they can quickly become contaminated with non-volatile fatty acids and waxy esters. The effect of this is that, as for splitless injection, these contaminants may provide sites for chemical degradation/adsorption of analytes.

The transfer method results in the analytes entering the separating column over a relatively long time period. Where analytes of interest are of similar boiling point to that of the carrying solvent this can result in peak broadening and peak splitting. This effect is not observed in the analysis of PCBs and OCPs since they generally have higher boiling points than the commonly used solvents.

2.2.1.4 Conclusion

Consideration of the above points indicate that on-column injection and splitless injection are suitable options for the analysis of environmental samples for organochlorine contaminants.

2.2.2 Choice of Column Parameters

The key requirement of the chromatographic process is that it should separate the components of interest. Chromatographic separations are based on the multiple partition of the analytes between two phases. In gas chromatography these two phases are the carrier gas and the stationary phase. The efficiency of the separations are affected by the choice of these phases and their physical dimensions e.g. length, internal diameter, and thickness. Literature searches have shown that a bewildering variety of columns are currently used for the separation of the target analytes in environmental matrices. Columns vary not only in stationary phase but in length and internal diameter. Recently 25, 30 and even 60m long columns with internal diameters from 0.1mm to 0.53mm and stationary phase thicknesses from 0.15 to 1.0µm have been used in multi-residue methods (de Boer *et al.*, 1992 (c); Holland & Malcolm, 1992; Lopez-Avila *et al.*, 1988 (a); Lopez-Avila *et al.*, 1988(b); Schantz *et al.*, 1993.).

2.2.2.1 Stationary Phase

The stationary phase is a thin liquid film coated or chemically bonded to the inside of the capillary column, analytes temporarily interact with the stationary phase. It is common practice to classify stationary phases according to their polarity:

(1) Apolar stationary phases : These interact with analytes essentially by dispersion forces. A typical apolar stationary phase is squalane. These phases are suited to packed column applications but have seen little use in capillary chromatography.

(2) Low Polarity stationary phases : Examples of these are methyl-, and alkyl-polysiloxanes. These are the most commonly used stationary phases in capillary chromatography. OV-1, CP-SIL 5 and DB-1 are examples of this class of stationary phase.

(3) Medium polarity stationary phases : Examples of these are phenyl methyl polysiloxanes. These are widely used in capillary chromatography. DB-5 and CP-SIL 8 are examples of this class of stationary phase.

(4) High polarity stationary phases: Examples of these are Cyanoalkyl substituted methyl polysiloxanes, polyethylene glycols or polyproylene glycols. These phases see wide spread use in both packed and capillary chromatography. CP-SIL 19 and Carbowaxes are examples of this class of stationary phase.

(5) Chiral stationary phases : Examples of these are crystalline phases with selectivity based upon molecular geometry. This class of stationary phase is not widely used. SB-Smectic is an example of this class of stationary phase.

The consensus of opinion of environmental analysts and recent international laboratory exercises (Wells et al., 1992.) is that a medium polarity stationary phase such as DB-5 or SE-54 should be used for organochlorine/PCB analysis.

2.2.2.2 Physical Dimensions

A decrease in the internal diameter or an increase in the length of a capillary column results in enhanced resolution power. Resulting technical/mechanical problems in injecting into very small diameter columns (<0.15mm) with high carrier gas pressure (>10-15 atm) produce selection problems between the theoretically ideal and the practical.

Results from inter-comparison exercises (Wells et al., 1992.) have clearly shown that reliable accurate data can only be achieved using capillary columns of a minimum length of 50m and a column id of not greater than 0.25mm. The film thickness should be between 0.2 and 0.4μm (Ettre 1985; Seferovic et al., 1986.). A thinner film results in a faster chromatogram but a loss in resolution at the beginning of the chromatogram.

2.2.2.3 Carrier Gas

The selection of carrier gases has been the subject of (Schomburg, 1990.) much discussion between chromatographers.

The present consensus is that hydrogen is the preferred carrier gas. It offers good resolution of compounds, even at a higher gas velocity, since the height equivalent to a theoretical plate (HETP) is relatively unaffected by the flow-rate above the optimum (de Boer et al., 1992(a); de Boer et al., 1992(b)). The resolution of components when using both helium and nitrogen declines as the gas velocity is increased. Helium may be used as an alternative to hydrogen, but the extremely high pressures with very narrow columns (0.2mm) cause practical difficulties. Helium may be used for columns with internal diameters >0.2mm although the resolution obtained will be less than with hydrogen. Nitrogen is not suitable for use in capillary GC as it significantly reduces the column efficiency at higher gas velocities.

2.2.2.4 Conclusions

A capillary column of medium polarity stationary phase, length at least 50 m, internal diameter ≤0.25mm, film thickness 0.25μm using either helium or hydrogen as carrier gas should be suitable for multi-residue applications.

2.2.3 Detection Techniques

The purpose of a detector is to quantitatively detect the compounds of interest. An ideal detector should be specific, sensitive, robust and give a linear response. There are a large variety of detectors available for the environmental analyst to choose from. The most common are flame ionisation, electron capture and the mass spectrometer. The advantages and disadvantages of common GC detectors as applied to multi-residue organochlorine pesticide analysis are discussed below.

2.2.3.1 Flame Ionisation Detector

The flame ionisation detector (FID) is the most widely used gas chromatographic detector. The FID is a non-selective detector which responds to all compounds that produce ions when burned in a Hydrogen-Air flame. This includes all organic compounds, although a few (e.g. formic acid, and acetaldehyde) exhibit poor sensitivity. The detection limit of this detector (0.5ppm) (Schomburg 1990.) is too high to allow determinations of organochlorines at environmental levels. This detector is more suited to screening extracts for contamination prior to detailed GC analysis.

2.2.3.2 Electron Capture Detector

The electron capture detector (ECD) is the most widely used selective detector in environmental analysis (Schwarzenbach *et al.*, 1984.). It responds primarily to molecules with functional groups with a high electron affinity. The ECD is very sensitive to most halogenated compounds and shows enhanced response to nitro compounds, highly oxygenated compounds, and some aromatic compounds. It has a low detection limit for many compounds.

The ECD cell contains ^{63}Ni, a radioactive isotope emitting high-energy electrons (β- particles). These undergo repeated collisions with carrier and make up gas molecules, producing about 100 secondary electrons for each initial β-particle.

Further collisions reduce the energy of these electrons into the thermal range. These low energy electrons may be captured by suitable sample molecules, thus reducing the total electron population within the cell. Un-captured electrons are collected periodically by applying short-term voltage pulses to cell electrodes. The cell current is measured and compared to a reference current, the pulse interval is then adjusted to maintain constant cell current. The pulse rate (frequency) rises when an electron capturing compound is passed through the cell. The pulse rate is converted to a voltage, non linearly related to the amount of electron capturing material in the cell.

The ECD is designed for use with a make up gas such as nitrogen or an argon /methane mixture. The ECD is not a linear detector. The response is related to the stereochemistry and number of electron attracting atoms or groups in the compound. To obtain precise results the detector must be calibrated with the target analytes in narrow concentration ranges where the response is close to linear. The ^{63}Ni foil is prone to contamination from non-volatile material and this can lead to a gradual reduction in sensitivity. The detector is also sensitive to changes in temperature and makeup gas flow rate. Gas flow rate needs careful optimisation, reducing the make-up gas flow rate increases the sensitivity, the non-linearity and the ease of contamination. The major disadvantage of the ECD is its lack of specificity. A large number of compounds containing halogens or electron capturing groups may be present in the final extracts. This may lead to the reporting of false positive results. In unusual circumstances it has been reported (Wells 1993.) that negatively biased results may be obtained when the analyte coelutes with an electron donating compound.

The ECD detector is cheap, readily available and easily used. These advantages far outweigh its disadvantages.

2.2.3.4 Mass Spectrometry

Mass spectrometry (MS) is a highly sensitive and specific technique suitable for use as a detector in environmental organic analysis. When combined with chromatography the result is a technique with high separating power and high discrimination. The technique has two modes of determination; electron impact (EI) ionisation and chemical ionisation which may be used to determine the chromatographically separated compounds. Each of these ionisation techniques is considered separately below.

Electron Impact Ionisation

GC-MS with electron impact (EI) ionisation is the most widely used technique by laboratories involved in environmental organic analysis. GC-MS with EI gives good sensitivity and reproducibility of the spectral data and can reveal structural information. A disadvantage of EI is that it does not always provide molecular weight information, as the molecular ion species, $[M]^+$, of many compounds is too labile to be observed in the mass spectrum. The reproducibility of EI data between different mass spectrometers using similar conditions, is generally good, so that rapid identification of spectra by comparison with mass spectral databases is possible. The limits of detection are in the low µg/L or µg/Kg level making the technique suitable for the determination of organic pollutants in water and solid samples. The sensitivity of GC-MS can be increased by one to two orders of magnitude by using only a few selected ions by the technique of selected ion monitoring (SIM).

Chemical Ionisation

GC-MS with either positive and/or negative chemical ionisation (PCI and NCI) is often employed in environmental organic analysis. Chemical ionisation is considered to be a 'soft' ionisation technique that employs a CI reagent gas, generally methane, but also isobutane and ammonia, at a source pressure varying from 0.1 to 0.2 torr.

PCI is routinely employed to obtain molecular weight information when conventional EI spectra has failed. Combined EI-PCI sources have been available for many years. For many compounds the sensitivity of PCI is similar to that of EI. The different ions obtained in PCI depend on the reagent gas used and compound employed.

The increasing use of GC-MS with NCI in environmental organic analysis is because many of the compounds of environmental interest contain electron-withdrawing groups and therefore stabilisation of the negative charge by electron capture is feasible. This results in a better signal in the negative ion mode. Ion forming reactions in the negative ion mode are of the following types:

(a) resonance and dissociative resonance electron capture
(b) ion molecule reactions
(c) radical-molecule reactions
(d) wall-neutral interactions
(e) ionisation-neutalization-reionization reactions

The formation of negative ions under electron capture negative ionisation is influenced by a number of factors including:

(i) the presence of reagent gas impurities
(ii) the charge density and composition of the plasma
(iii) long ion source residence times and
(iv) elevated pressure

The advantages of this technique for environmental analysis are selectivity and sensitivity, the disadvantages are the lack of structural libraries and the relative complexity of the analytical system.

High sensitivity, good precision (in comparison to MS), relative ease of use and low cost have made ECD detection the method of choice for the routine determination of a wide range of organochlorine compounds in environmental samples. However, ECD detection gives no structural information and it is recommended that a portion of results be confirmed by a MS technique.

All of these detectors rely up on accurate calibration. Numerous interlaboratory exercises (Wells & Kelly 1991; de Boer *et al.*, 1992(b); Wells & de Boer 1994.) have shown that the preparation, storage and correct use of calibration solutions is an often overlooked yet critical step in the production of accurate analytical data.

2.2.3.4 Conclusion

The electron capture and mass spectrometry (using both electron impact and negative chemical ionisation) are detectors suitable for environmental multi-residue applications.

2.3 Extract Clean-Up Techniques

Clean up is the term used in pesticide residue analysis for the isolation of the selected analyte from potentially interfering coextractives. The main requirement of the clean up is to effectively remove all of the coextractants from the extract without degrading or removing the selected analytes.

Coextractives fall into the following three categories :

(1) Gross contaminants e.g. lipids, sulphur, cartenoids and pigments.

(2) Interfering trace pollutants, e.g. PCBs interfering in the determination of trace quantities of organochlorine pesticides.

(3) Coeluting compounds not detected directly by detector but producing erratic response in the detector, e.g. micro quantities of phthalates reducing ECD response to coeluting PCBs.

Cleanup procedures can have disadvantages. These include loss of analytes, introduction of contaminants, restriction of target analytes and an increase in time cost of analysis. The commonly used cleanup techniques in multi-residue pesticide analysis are discussed below.

2.3.1 Non - Destructive Clean-up Techniques

2.3.1.1 Normal Phase Adsorption Chromatography

This is the most widely used clean-up method and forms the basis of many standard methods for low polarity contaminants, e.g. PCBs. In this method polar interferences are retained from the extract by an adsorbent whilst analytes of interest are recovered by graded elution with a solvent. The adsorbents most extensively used in residue analysis are silica, alumina, and florisil.

Silica

Silica is the most widely used chromatographic adsorbent. Silica is a typical all purpose polar adsorbent and is commercially available in a wide variety of forms. Silica is also frequently known as silica gel and silicic acid. It has the general formula is $SiO_2 * H_2O$ and is an amorphous porous solid made up of three dimensional siloxane structures with polar surface silanol, Si-O-H groups. Silica can be manufactured in a wide variety of pore diameters and surface areas: variation of solution pH during the acid gelation of sodium silicate yields silicas with surface areas varying from about 200 (pH $\cong$10) to 800(pH $\cong$4) m^2/g. The surface of silica is weakly acidic (pH 3-5), and there is a tendency towards preferential adsorption of strongly basic samples.

The adsorption sites on silica are the surface hydroxyl groups, these interact with the adsorbed molecules by hydrogen bonding; the adsorbate molecule normally functions as an electron donor. Three different types of hydroxyl groups (bound, free, and reactive) on the surface of silica give rise to differing surface activities. The percentage of any one type present on a silica can be greatly influenced by variations in activation temperature and the % water deactivation. Hence variations in procedures for preparing silica allow the analyst to control the activity of the silica. The activity can also be controlled by impregnation with different chemicals, e.g. silver nitrate and sodium sulphite. In general silica is the first choice general purpose adsorbent demonstrating a strong activity for polar molecules, with only slight activity towards differing degrees of aromaticity.

Alumina

Alumina is the second most popular adsorbent used in column chromatography. Alumina is also a typical all purpose polar adsorbent and commercially available in a wide variety of forms. There are several hydroxides and oxide-hydroxides, stable at only low temperatures. Upon being heated at 200 - 600 °C, they are converted into the so called low-temperature alumni's, which occur in several different crystal forms (γ, η, χ, ρ). At temperatures of 900 - 1000 °C the latter are in turn converted into high temperature alumni's, which again include several crystalline modifications (θ, δ, κ). When heated to above 1100 °C, all alumni's are transformed into α-alumina. α-alumina is chromatographically inactive (Snyder 1968). Commercial alumni's for adsorption chromatography are generally impure alumina. To achieve group separation and removal of co-extracted materials alumina is used in a wide number of forms. These include different mesh sizes, levels of activity and impregnation with compounds such as silver nitrate. Similar to silica, alumina demonstrates a high degree of activity towards polar molecules. However, in contrast to silica, alumina demonstrates good selectivity towards different degrees of aromaticity.

Florisil

Florisil is one of the oldest materials used in the cleanup of pesticide residue extracts. It is a mixture of several inorganic oxides with SiO_2 and MgO as the main components. It is a general purpose polar absorbent. Its activity is generally seen as intermediate between alumina and silica. Its surface is strongly acidic and basic compounds are more strongly retained than on alumina or silica.

Many classes of compounds chemisorb onto florisil, this property initially seen as a disadvantage in its use as chromatographic material, enhances its use as a clean-up material.

2.3.1.2 Reverse Phase Chromatography

These sorbents are derived from porous silica, modified by reacting the surface hydroxyls with halo- or alkyl-siloxane derivatives. This bonding imparts adsorptive characteristics to the silica which is primarily a function of the bonded phase. Recently a number of different types of these phases have become available and a full discussion of these is given in section 2.4.1.2. The phases which

have seen the greatest application to environmental sample cleanup are aminopropyl, C8 and C18. The aminopropyl phase takes advantage of the ability of compounds to hydrogen bond with the bonded phase. Thus monoglycerides (with two free hydroxyl groups on the glycerol chain) can form a strong interaction with the hydrogen bonding sorbent whilst triglycerides with no free OH groups can form only weak polar interactions made possible through the polarity of the ester linkages. C8 and C18 phases take advantage of interactions based upon Van Der Waal's dispersion forces between the carbonaceous component of the isolate and the sorbent. These linkages are discussed more fully in section 2.4.1.2. The main advantages offered by these sorbent clean-ups are speed and ease of use. These phases are available in commercially prepared cartridges ready for use. Traditional polar adsorbents are more usually prepared and activated/deactivated in the laboratory and used in manually prepared glass columns. Recently florisil has become available in prepared cartridges.

2.3.1.3 Gel Permeation Chromatography

Gel permeation chromatography (GPC) is the separation of components of a mixture using molecular size exclusion. GPC is generally conducted using porous polymeric beads made from materials such as a styrene divinylbenzene co-polymer. These beads swell up into a gel when placed in a suitable solvent and are usually packed into one or more columns. The beads contain a known size range of porous openings. Molecules percolate through the column at different rates, based upon their ability to penetrate into the pore spaces. The separation is based primarily upon size exclusion chromatography (SEC) The larger molecules are excluded from the pores and travel between the gel particles to emerge from the column first. Smaller molecules enter the gel particles through the pores and travel through the gel particles spending much of their time within the gel pore regions emerging last from the column. Although based primarily on SEC mechanisms this technique is also influenced by affinity interactions between the solutes, the mobile phase and the polymer gel particles. During GPC solute molecules may demonstrate affinity for the electronic structure of the polymer gel through π-(and other donor/acceptor) interactions. As a result of this retention times differ from those expected based purely on size exclusion behaviour. Generally increasing aromaticity increases retention on the column.

GPC has been used for over 30 years (Kuel & Leonard, 1963) as a clean-up method in residue analysis. GPC has been shown to be an excellent cleanup method for the removal of matrix co-extractives for a wide variety of sample types and analytes including: fish (Ribick 1982.), animal products (Holestedge _et al_ ., 1991), fruit and vegetables (Roos _et al._, 1987) and sediments (Krahn 1988). GPC has several advantages over other clean up methods: The method is non destructive and can be used to isolate less stable analytes such as organophosphorus pesticides. The technique is not functional group or polarity specific.

The two main disadvantages of GPC are, the suppression of non size exclusion interactions between the analytes and the GPC by chlorinated solvents and the difficulty in removing some interferences of similar molecular weight to the analytes resulting in the requirement for a second clean-up.

One of the major obstacles preventing wide spread use of GPC is the high initial capital cost of purchase of GPC columns and liquid chromatography systems capable of automated fraction collection.

2.3.1.4 Sweep Co-Distillation

A solvent extract of a sample is injected into a heated glass tube packed with glass wool. Using nitrogen acting as a carrier gas the mixture is swept through into a collection vessel containing a solvent such as hexane. The sample co-extractives are deposited onto the glass wool, whilst the pesticides are volatilised and collected in a concentration tube. This technique is not widely used because it has poor reliability due to sample loss caused by chemical degradation and absorption.

2.3.1.5 Dialysis/Partitioning

Polyethylene film of pore size ca. 50µm can be used to dialyse an organic extract to isolate pesticides from fat (Huckins *et al.*, 1990). A sample extract containing up to 10g of fat is placed in pre-washed polyethylene bag, the bag is placed in a beaker containing cyclopentane. Approximately 95% of the pesticides will dialyse into the surrounding solvent. The polyethylene has a molecular mass cut-off of around 500 Daltons and acts as a static size-exclusion membrane. This method is simple and effective for removing bulk amounts of fat. Large amounts of solvent are required, and 4-5% of fat dialyses into the solvent necessitating further clean-up steps. This need for additional clean-up and the possibility of contamination from plastics have meant that this promising technique has seen little use.

2.3.1.6 Conclusions

GPC is the clean-up technique most suited to the removal of gross contaminants, however, as indicated the equipment required for GPC is expensive. Although GPC is well suited for the removal of gross contaminants it is unable to separate interfering trace pollutants or prevent coelution of the analytes with compounds of similar molecular weights e.g. phthalates, or humic acids. GPC must be used in conjunction with another cleanup technique to remove these interferences. The equipment necessary to undertake GPC clean up was not available for this study. Normal phase chromatography using alumina, modified alumina, and florisil as the adsorbents and reverse phase chromatography using aminoproyl bonded silicas were the techniques available for use which are most suited to the removal of gross contaminants. Normal phase techniques are also appropriate for the removal of polar co-contaminants such as humic acids and phthalates. Normal phase chromatography with silica as the adsorbent is the technique most suited for separation of analyte classes e.g. PCBs from OCPs.

2.3.2 Destructive Clean-Up Techniques

Clean-up by destructive chemical reactions offer a useful alternative to the chromatographic clean ups discussed earlier. There are three main types of chemical clean-ups: chemical combination, acid and alkaline treatments.

2.3.2.1 Chemical Combination

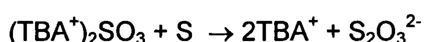
Elemental sulphur is present in most soils, sediments and sewage effluents and is sufficiently soluble in most common organic solvents that a proportion will be extracted from the sample. Sulphur and simple sulphur compounds give a response with all detector types and may mask early eluting compounds. Sulphur compounds can also irreversibly poison an Electron Capture Detector (ECD). Because of these problems at the detection point sulphur removal is a key step in any multi-residue method applied to sediments and sewage effluents. At present there are four main methods of sulphur removal:

(1) Reaction with Mercury or a Mercury amalgam to form insoluble Mercury Sulphide. This is the oldest and possibly the most effective method of sulphur removal. However, due to the potential health risks from exposure to mercury and its compounds this method is rarely practised.

(2) Reaction with copper to form copper sulphide. Removal of sulphur with copper requires the metal surface to be clean and reactive. If small pieces of copper wire are used this can be achieved by rubbing the metal surface with emery paper before exposure to the extract. Larger amounts of sulphur can be removed by the use of copper powder. Before use the powder must be activated by rinsing with dilute Hydrochloric Acid. This method is particularly suitable for use with sediments that are to be soxhlet extracted as the copper treatment may be applied as part of the solvent extraction process. This is achieved by adding the copper to the solvent collection flask.

(3) Reaction with a solution of sodium sulphite in tetrabutyl ammonium sulphate known as Jensen's Reagent (Jensen *et al.*, 1977). The extract is shaken with a tetrabutyl ammonium sulphate solution which has been saturated with sodium sulphite in the presence of isopropanol.

The tetrabutylammonium ion forms a lipophilic ion pair with the excess sulphite. The isopropanol is a very good solvent for such ion pairs and favours the solvation of the ion pair in the nonpolar phase. The sulphite is brought into contact with the elemental sulphur causing a momentary reaction forming the highly aqueous soluble thiosulphite. The equation for this is given below.



$$(TBA^+)_2SO_3 + S \rightarrow 2TBA^+ + S_2O_3^{2-}$$

When the isopropanol, the tetrabutylammonium sulphate and the extract are mixed, some sodium sulphite precipitates. In an excess of sulphur, all the sulphite is converted to the water soluble thiosulphate. To ensure complete removal of all the sulphur more sulphite must be added until a precipitate remains.

2.3.2.2 Treatment with an Alkali

Triglycerides and wax esters can be removed by saponification with 20% potassium hydroxide. This method is extremely efficient at the removal of fats and lipids. It is an extremely harsh treatment and is an unsuitable extract clean-up except for the most chemically resistant analytes. Examples of analyte degradation are the hydrolysis of OCPs and the dechlorination of DDT and Hexachlorohexane. Due to their chemical stability the technique has been successfully applied to the determination of some PCBs. However, even with these persistent contaminants care must be taken as the more highly chlorinated PCBs are prone to loss of chlorine when the reaction is undertaken at too high a temperature (Wells & Echarri 1992). The severity of this treatment has resulted in its use as a confirmatory technique (Chau 1982) for active contaminants. In this technique extracts are deliberately excessively hydrolysed and the resulting extract analysed for the degradation products.

2.3.2.3 Treatment with an Acid

Treatment by sulphuric acid is another harsh chemical method used to remove co-extractants by dehydration and oxidation reactions. This method is suitable for only the most robust contaminants. All compounds containing an oxygen bridge will be degraded by this method, e.g. dieldrin, endrin. Two approaches are used in this treatment, the first involves shaking the sample extract with concentrated sulphuric acid. Care must be taken to ensure that the extract is in an alkane solvent and not acetone or acetonitrile which would react violently with the acid. The second approach is to load up to 40%w/w of sulphuric acid onto a silica support. The sample can then be denatured by passing through a column of the acid impregnated silica and eluting with dichloromethane. Both approaches have severe draw backs. Solvent treatment can produce a violent reaction producing large amounts of heat also the separation between the extract and acid can become unclear leading to losses. In the second approach the preparation and disposal of the acidic/silica presents a possible safety hazard. The silica particles are extremely fine and become airborne very easily. These particles are coated with concentrated sulphuric acid and care must be taken to avoid inhalation or ingestion of these particles. All work using acidic/silica should be carried out in a fume cupboard.

Despite the potential hazards and the restricted compound application this technique has gained widespread use. The main reason for this acceptance is the large amount of fat which can be removed by this method. A 50g silica column can remove up to 10g of fat from an extract (Hess *et al.*, 1995). No other clean-up method can offer this scale of fat removal. In applications such as the determination of non-ortho substituted CBs, where detection limits are of the utmost importance sulphuric acid treatment will continue to be the first choice clean-up.

2.3.2.4 Conclusions

Saponification and Sulphuric acid treatment are the most powerful clean up techniques, but, these aggressive treatments destroy a large number of potential analytes. Use of these techniques severely limits the range of analytes and because of this they are not suitable for multi-residue methods. Both treatment with copper powder and tetrabutyl ammonium sulphite appear suitable for the removal of sulphur in multi-residue methods.

2.4 Extraction Techniques

The level of contaminants in most environmental samples is such that the contaminant residues must be concentrated from the bulk of the sample matrix by extraction. Ideally the extraction step should remove all of the analytes and nothing else. In practice this is an unrealistic goal and the optimum is to maximise the extraction efficiency of the analytes while minimising the extraction of non target compounds.

The choice of extraction technique is determined by the sample matrix. In environmental studies there are 3 main types of matrices, a) aqueous based matrices e.g. surface waters, and sewage effluents, b) sediments, and c) biological materials. Sediment and biological samples are solid matrices which have similar extraction problems and therefore may be considered together. Water based matrices are considered separately.

2.4.1 Extraction of Aqueous Matrices

2.4.1.1 Liquid-Liquid Extraction

Liquid-liquid extraction is the most widely recognised technique in trace organic analysis. It involves shaking or mixing the sample with an organic solvent to partition the analytes between the two liquids. This may be achieved by manual shaking in a sample bottle or a separatory funnel. More recently mechanical shaking devices, vortex mixers and commercial continuous liquid-liquid extraction systems have been used since they give a more reproducible and efficient extraction. In all but continuous systems, multiple extractions (typically two or three) are carried out to maximise the recoveries of the analytes. Hexane, and dichloromethane are the two most commonly used solvents. Both are readily available in high purity and a wide variety of organic compounds exhibit a high solubility in them. In addition both are relatively immiscible with water, and their volatility ensures that any subsequent evaporation steps are relatively simple. The addition of a salt such as sodium chloride or sodium sulphate to reduce the water solubility of the analytes and hence improve the extraction recovery is also common place. The wide use of liquid-liquid extraction owes much to its simplicity as this technique has many shortcomings.

Liquid-liquid extraction is comparatively expensive to carry out; manual systems are labour intensive, time consuming, use large volumes of solvents involving exposure of staff to possibly hazardous solvents, and can give rise to erratic recoveries through the formation of emulsions.

Despite these drawbacks liquid-liquid extraction continues to be the most commonly used extraction technique for aqueous samples.

2.4.1.2 Solid Phase Extraction

Solid Phase Extraction (SPE) chromatography, is accepted as the removal of analytes from a liquid sample as it passes through a solid adsorbent, followed by subsequent elution of those analytes with a small amount of solvent. A wide variety of materials utilising a range of different surface characteristics and adsorption mechanisms have been successfully applied to this type of approach. These include charcoal, cellulose fibres, polymers, and bonded silica gels.

Charcoal was the first sorbent to be used for the extraction of pesticides from water (Junk *et al.*, 1987). The advantage of this material was the high retention of polar pesticides and their metabolites (Bavaloni *et al.*, 1980; Farran & Pablo, 1987; Di Corccia & Marchetti, 1991). Polymers have also been extensively used as sorbents since the late sixties. Commonly used polymers are: styrene-divinylbenzene copolymers such as XAD-2 or XAD-4 (Gomez-Belinchon *et al.*, 1988; Richard & Junk, 1986; Schultz *et al.*, 1988), acrylate polymers such as XAD-7 and XAD-8 (Dietrich & Millington, 1988), 2,6-diphenyl-p-phenylene oxides such as tenax (Leuenberger & Pankow, 1984) and polyurethane foams (Wells & Johnstone, 1978).

There are two major mechanisms for analyte retention on a solid support, adsorption and partitioning. The materials listed above all use adsorption mechanisms, however the development of surface-modified materials for liquid chromatography has led to the development of a range of materials which rely on partition mechanism for analyte retention.

The most commonly encountered partition SPE sorbents are based on porous silica, the surface of which has been modified by reacting the surface hydroxyl groups with halo- or alkoxy-silyl derivatives. The high surface coverage achieved during this bonding stage imparts an adsorptive characteristic to the bonded silica that is primarily a function of the phase bonded to the silica surface. Due to steric hindrance during the bonding reaction, some silanol groups will be inaccessible to the bonding reagent, this leaves a small number of free adsorptive sites and accounts for the small amount of adsorption behaviour demonstrated by some of these materials.

The selectivity of bonded silica sorbents is a function of both the solvated bonded phase and the solvents used to elute compounds from the extraction columns. Commonly encountered bonded silicas are listed in Table 1.

Table 1 Commonly encountered bonded silicas

C18	Octadecyl
C8	Octyl
C2	Ethyl
PH	Phenyl
CN	Cyanopropyl
2OH	Diol
NH2	Aminopropyl
SCX	Benzenesulphonic Acid

Non-polar interactions

Non-polar interactions are based upon Van Der Waals dispersion forces that occur between the carbonaceous component of the isolate and the sorbent functional group. The main non-polar sorbents are C18, C8, C2 and phenyl although all bonded silicas display some non-polar interactions. Non-polar sorbents and C18 and C8 in particular are the phases most commonly used to isolate pesticides from aqueous matrices in multi-residue methods.

Polar interactions

Retention of polar compounds by one of the polar sorbents occurs in a non polar environment in a solvent such as hexane. Retention mechanisms include hydrogen bonding, dipole-dipole, induced dipole and π-π interactions. Elution of polar compounds from the polar sorbents is achieved using a polar solvent mixture such as methanol and acetonitrile. This technique has seen limited application for the isolation of pesticide residues, but has seen more use as a clean-up technique.

Ionic interactions

Isolate retention by ion exchange is generally from an aqueous sample. Retention and elution of isolates from the column is by use of pH control and buffer concentration. In general the extraction of an isolate using an ion exchange mechanism is capable of producing a very clean extract. The technique is limited to those compounds which contain an ionisable group.

2.4.1.3 Conclusion

Both of these techniques are suitable for the extraction of a wide range of target analytes from aqueous samples.

2.4.2 Extraction of Solid Matrices

2.4.2.1 Soxhlet Extraction

Soxhlet extraction is used for the isolation of non-polar and semi-polar trace organics from a wide variety of matrices.

The choice of solvent is critical with all the criteria outlined in liquid-liquid extraction to be met with additional regard to the solid matrix acting as a chromatographic material. Mineral components have the properties of a normal phase material while organic components act as a reverse phase material. The former require a polar solvent and the latter an apolar solvent to obtain the highest elution potency. Although used for many years there is now clear evidence to suggest that the use of non-polar solvents on their own is not suitable for the recovery of PCBs and OCPs from both lean and fatty tissues (de Boer 1988).

The main advantages of soxhlet extraction is that multiple systems are possible, leading to batch processing of samples. The extraction requires low operator labour intervention, proceeding unattended once it has been initially set up. The apparatus is relatively inexpensive and widely available. The main disadvantages are the large volumes of solvent required (100-300 mls/ sample) and possible losses of volatile analytes.

Soxhlet extraction has become the most widely used extraction technique for solid matrices.

2.4.2.2 Blending and Ultrasonic Extraction

In this technique a wet or dry sample of sediment or tissue is repeatedly equilibrated with a solvent or a mixture of solvents by ultrasonication or blending. The resulting mixture is usually separated by filtration or centrifugation. The extraction is repeated two to three times to ensure complete removal of the analytes. Ultrasonication has become popular in recent years with the development of more powerful ultrasonication devices. These techniques are labour intensive, difficult to automate and expose operators to potentially hazardous solvent aerosols.

2.4.2.3 Column Extraction

This is a relatively simple procedure where a sample of sediment or biota is ground with a drying agent such as sodium sulphate and packed into a large glass column (100cm * 10cm id). Solvents such as dichloromethane are added to the top of the column and allowed to soak for a set period of time (typically 30 mins). The solvent is then slowly drained into a collection vessel. This procedure of soaking and draining is repeated two to three times or until all the contaminants are extracted. This method is easy to set up and requires no specialised equipment but needs a large amount of space and large volumes of solvent. Because of the solvent volumes care must be taken to control the extraction blanks. These restrictions have limited its use to the extraction of large samples.

This technique is labour intensive, difficult to automate and expose operators to potentially hazardous solvent aerosols.

2.4.2.4 Supercritical Fluid Extraction.

Until recently supercritical fluid extraction (SFE) had been used mainly for large scale applications such as the decaffenation of coffee. It is increasingly being used for analytical scale sample extraction.

A fluid is said to be supercritical when both its temperature and pressure are above their critical values. The critical point is defined as the temperature and the pressure above which the substance is neither a gas nor a liquid but posses the properties of both. At temperatures and pressures above the critical point the substance cannot be liquefied regardless of the pressure exerted on it, and is called a super critical fluid.

Supercritical fluids have several properties that make them excellent extraction solvents. The viscosity of a supercritical fluid is 5-20 times lower than that of ordinary liquids, consequently they can penetrate the pores and interstices of the matrix better. Supercritical fluids have densities 100-1000 times greater than those of gases, which gives them solvating power close to that of liquids. In addition they are generally non flammable, non toxic fluids such as CO_2 which can easily be vented to atmosphere after decompression, removing the need for costly time consuming solvent evaporation steps.

SFE is carried out by placing the sample in contact with a static supercritical fluid in a closed container or by percolating a supercritical fluid through the sample. The analytes are removed in the supercritical fluid from which they must be isolated before further analyses. Isolation is accomplished by decompression of the fluid into a liquid trap or onto a solid trap. SFE is a recent evolving technique in which the effects of many experimental factors such as pressure temperature and modifiers are only beginning to be understood. Instrumentation and applications of SFE are being developed at an increasing rate and while not yet being a robust routine tool it is clear that in the future it will become a powerful tool that will be utilised by many analysts.

2.4.2.5 Conclusion

Soxhlet and supercritical fluid extraction are the techniques most suitable for extracting a wide range of target analytes from a variety of sample matrices. Unfortunately only soxhlet extraction was available for evaluation in this study.

2.5 Sample Preservation and Storage

For many practical reasons it may be not possible to start the analysis of an environmental sample immediately after sample collection. Many chemical, physical and biological processes may occur in the sample between collection and analysis, therefore, it is essential to assure the samples integrity from collection to analysis. Preservation of the sample is as important as the analysis in ensuring that the determined concentrations accurately reflect those in the sample. The techniques for the preparation and storage of samples are matrice dependent and therefore water, sediment and biota samples are considered separately.

2.5.1 Aqueous Samples

The main factors affecting the stability of analytes in water samples include the character of the sample, the sample container, and the conditions of storage (temperature, light, use of preservatives, time of storage). Chemical and biological changes inevitably continue after sample collection and may produce losses and/or degradation of target analytes. Following sampling, rapid changes in temperature and pH (Jeannot 1994.) may occur, dissolved oxygen and volatile compounds (Keith 1988.) may be lost, lipophilic compounds may be irreversibly bound to the sample container (Erickson 1986) and ambient light may initiate photo-chemical reactions (Chiron *et al.*, 1993; Keith 1988; Novotny *et al.*, 1981). Regulatory authorities throughout the world have made recommendations for the containers, preservation techniques and holding times for organochlorine pesticides and PCBs in water these are summarised in Table 2.

From these and standard chemical texts a number of general practices for minimising changes in water samples can be summarised.

(1) Biological degradation can be minimised by chemical additions pH modifications or temperature control.

(2) Volatilisation can be minimised by using sealed containers without a headspace and cooling.

(3) Chemical reactions can be can be minimised by chemical additions: sodium sulphite or sodium thiosulphite to prevent oxidation, pH modifications, cooling, or amber glass to prevent photochemical changes.

(4) Adsorption can be minimised by choice of container material (almost always glass for organics analysis) .

These wide variety of preservation practices illustrate the fact that there are no universal rules to prevent changes in water samples.

Surprisingly with such a wide variety of techniques being routinely used few studies have been conducted to investigate which practices are effective and necessary. A series of detailed studies was conducted by the United States Environment Protection Agency (USEPA) during the development of methods for pesticide analysis of well-water samples (Munch et al., 1990; Munch & Frebis, 1992). These studies suggest that collection in glass containers and refrigeration within a few hours of collection will preserve organochlorine compounds for at least 14 days.

Table 2 Recommended Sampling Containers, Preservation Techniques and Holding Times for Aqueous Samples for Organochlorine Pesticide Analysis.

Compound Class	Regulatory Authority	Container	Preservation	Holding Time
OCPs	US EPA	Glass	Refrigeration, adding 1ml of 10mg/ml $HgCl_2$	7 days for extraction, 40 days after extraction.
OCPs	APHA	Solvent rinsed Glass	Refrigeration, adding 100mg of ascorbic acid/l	7 days for extraction, 40 days after extraction
OCPs	ISO	Glass	Refrigeration adding extraction solvent	7 days
OCPs	ASTM	Amber Glass	Refrigeration	Not Stated
OCPs	UKSCA	Glass	Refrigeration	Solvent extracts can be stored for months
PCBs	US EPA	Glass	Refrigeration	7 days for extraction, 40 days after extraction
PCBs	APHA	Glass	Refrigeration, pH range 5 to 9	7 days for extraction, 40 days after extraction
PCBs	ASTM	Amber Glass	Refrigeration	14 days for extraction

2.5.2 Biota

All of the factors affecting analyte stability in aqueous samples also apply to a lesser or greater degree to samples of biota. Unfortunately, the recommendations and guidelines for biota are even more muddled than for aqueous samples. As far as the author is aware no definitive studies have been conducted to establish storage conditions/time for which biota samples can be stored without problems. However, some experience may be gained from laboratories engaged in ongoing monitoring programs and the recommendations of bodies such as the International Council for the Exploration of the Seas (ICES).

It is generally accepted that in order to prevent tissue decomposition biota should be dissected immediately upon capture. Often this is not possible as biota are caught and killed in the field with no facilities suitable for dissection or treatment. Where this occurs the samples should be stored in a freezer at -20 °C prior to dissection. Individual organisms should be stored in solvent rinsed aluminium foil, placed in plastic bags, and frozen quickly.

Obtaining tissue samples or specific organs e.g. liver for chemical analysis, from each specimen also presents problems. The dissection must not affect the contaminant levels in the tissue. The dissection tools must be free from the analytes and must not interact with the analytes. Stainless steel dissection tools are recommended for trace organic analysis. Frozen tissues express a substantial amount of fluid (up to 20% of tissue mass may be lost, ICES 1991) on thawing. This fluid may or may not contain appreciable amounts of the analytes. It is necessary to keep the specimen partially frozen during dissection to minimise such fluid losses. It is important to remember that certain tissues do not comprise of a single cell type and that a gradient in analyte concentration may be present along one axis of the tissue due to a gradient in a cellular constituents such as fat. It is recommended that complete organs are sampled and that thorough homogenisation be carried out before sub samples are selected. At this stage samples can be refrozen for long term storage or dried prior to storage.

Drying samples prior to storage and analysis offers several advantages to the analyst; a) It reduces the amount of freezer space required reducing costs and easing sample tracking/handling and, b) the analysis of dry samples reduces the errors associated with variable water content of samples (How wet is wet ?) and allows the use of non water miscible extraction solvents e.g. DCM and Hexane. These advantages favour the drying of samples prior to analysis/storage. Three main methods of drying are currently practised: 1) Oven drying, 2) freeze drying, and 3) chemical drying.

Oven drying involves placing the sample in an oven at a relatively low temperature e.g. 40 °C and over a period of days allowing it to obtain constant mass. Dry samples may be stored in sealed amber glass containers. The advantages of this technique are that it is cheap and uses little or no specialised equipment. The disadvantages of this technique are:

i) analytes may be lost due to evaporation
ii) there is a risk of cross contamination if "dirty and clean" samples are dried in the same oven
iii) the samples will smell strongly during the drying process. These disadvantages outweigh the advantages.

Freeze drying is widely practised for a large variety of environmental samples. The process can be automated and lends itself to processing large numbers of samples. As with oven drying care must be taken to avoid losses of volatile components and cross contamination. No definitive studies have been conducted on the optimum freeze drying conditions. Once dry the samples can be stored prior to extraction. This is the most convenient technique to use for drying samples although studies to investigate possible analyte losses must be undertaken prior to its routine use.

Chemical drying can be performed by grinding with anhydrous sodium or magnesium sulphate. Historically this is the most widely practised drying technique. It is time consuming and cannot be automated. When the sample has been dried it should be extracted within 24 hours. Errors may be introduced if the drying agent is not very pure, and some analytes may be irreversibly adsorbed. This technique remains the benchmark to which others are compared.

2.5.3 Sediments

As for biota and water samples, the preservation and storage of sediments remains an area where very few studies have been conducted. Glass jars with PTFE or solvent rinsed aluminium foil lined caps are the preferred storage containers. Samples are usually stored refrigerated at -20 °C and are typically considered stable for 1-6 months. For the same reasons as described for biota the drying of sediments prior to analysis is an attractive option. The three methods outlined previously for drying biota are also used for sediments. Freeze drying is the most convenient and easily used method.

A problem with all drying methods of sediments is that significant aggregation occurs. To avoid errors from this if particle size analysis is to be carried out it should be done prior to drying.

Sediments are usually taken as material with a grain size <2 mm. After drying it is normal practice to lightly grind the sample to break up any aggregates which may have formed during the drying process. The sample is lightly sieved through a 2mm mesh sieve to remove pebbles, coarse organic fibres, shell or plant debris which may be present in the sample. While conducting these steps great care must be taken to avoid cross contamination of the sample or adsorption of the analytes.

2.6 Conclusions

From the methods of analysis reported in this work, it is clear that a wide variety of methods of analysis are used for the determination of organochlorine compounds. At present these methods are usually specific for a limited number of target analytes such as PCBs or DDT type compounds. In addition many of these methods developed in an ad-hoc manner over a period of time, comprehensive studies which fully investigate these procedures and identify the steps which are crucial to the accuracy and reproducibility of the methods, have rarely been conducted. Very few methods have been published for the determination of modern pesticides such as the pyrethroids in environmental matrices. There is a clear environmental need to determine the environmental concentrations of both " traditional " contaminants such as PCBs and more modern contaminants such as permethrin. The most effective method of obtaining this information is to determine large numbers of contaminants alongside each other in multi-residue analysis schemes.

From the previous review of published analytical procedures for the determination of organochlorine contaminants the following techniques have been identified as worthy of investigation for use in a multi-residue method for the determination of organochlorine and synthetic pyrethroid contaminants.

Capillary gas chromatography with a medium polarity column of at least 50m length, an internal diameter of ≤0.25mm, film thickness 0.25μm using either helium or hydrogen as carrier gas was selected as the most appropriate methods of separation prior to detection. On-column and splitless were selected as the most appropriate methods of sample introduction for the gas chromatograph. Electron capture and mass spectrometry using both electron impact and negative chemical ionisation were selected as the most appropriate detectors for environmental applications.

Gel permeation chromatography was recognised as potentially the most useful cleanup technique, however, it was recognised that the need for specialised equipment would restrict the use of this technique. Adsorption chromatography with normal and reverse phase adsorbents was selected as the technique most suited for use in multi-residue analysis schemes. Destructive clean-up techniques based on chemical reactions with strong acids or alkalis can remove large amounts of interferences, however, these aggressive treatments destroy large numbers of potential analytes and severely limit the range of target analytes. The only chemical treatments selected as appropriate for use in a multi-residue method were copper powder and tetrabutyl ammonium sulphite.

Liquid-liquid extraction is the most widely recognised technique in trace organic analysis. It's use with both dichloromethane and hexane was considered appropriate for the extraction of a wide range of analytes from aqueous samples. Solid phase extraction was also identified as a cost effective method of extracting a wide range of analytes from aqueous samples. Soxhlet and supercritical fluid extraction were identified as the most appropriate method of extraction from solid matrices. Unfortunately only soxhlet extraction was available for evaluation in this study.

Chapter 3

Optimisation of Analytical Techniques

3.1 Introduction

The aim of this section of work was to develop a method suitable for use in regulating a range of persistent organochlorine chemicals which are of environmental concern and discharged from wool processing and textile industries.

The analytical techniques highlighted in the previous section as suitable for use in modular based multi-residue methods are in this section sequentially investigated and optimised. The most robust and effective techniques are selected and combined into modular methods suitable for use in a range of environmental matrices. Routine analytical quality control procedures for these methods are established and the methods fully validated.

As indicated in the introduction the range of target analytes for these methods was primarily influenced by two factors: 1) the need to determine environmental concentrations of mothproofing chemicals to enable control of discharges from the textile and wool processing industries, 2) the need to comply with European legislation (principally the "dangerous substances directive" and the Red List) and monitor environmental concentration of priority pollutants. Acknowledging the limitations of the analytical techniques outlined previously, the compounds listed in Table 3 were selected as target analytes to be determined.

Table 3 Target Analytes

Compound	Compound
cis permethrin	PCB 28
trans permethrin	PCB 52
dieldrin	PCB 101
aldrin	PCB 118
endrin	PCB 138
PP DDT	PCB 153
PP DDD	PCB 180
PP DDE	PCB 105
OP DDT	PCB 156
OP DDD	bifenthrin
OP DDE	Alpha endosulfan (Endo A)
α HCH	Beta endosulfan (Endo B)
β HCH	hexachlorobenzene (HCB)
γ HCH	hexachlorobutadiene (HCBD)

As per the previous section procedural steps were investigated in reverse order both because features in some prior steps are controlled by limitation of subsequent steps, but also as robust method of detection was necessary before steps such as cleanup and extraction could be investigated.

3.2 Optimisation of Determination

As outlined in section 2.2 literature studies indicate that gas chromatography using a medium polarity column with final detection by mass spectrometry or electron capture is the most appropriate means of determining organochlorine environmental contaminants.

For this study the following analytical instrumentation was initially available for use:
Shimadzu 14 A GC fitted with AOC - 14 Autosampler, Split /Splitless and On-Column injectors and an Electron Capture Detector, a Trio 1 Mass Spectrometer fitted with a HP5890 Gas chromatograph, and a Vinten Scientific TLAS chromatography data collection and integration software.

Electron capture was selected as the method of detection to be initially optimised. The specific components of the determination requiring optimisation in the GC/ECD determination were detector response, injection technique and gas chromatographic separation. The optimised injection technique and chromatographic separation could also then be applied to mass spectrometric detection, thus allowing optimisation of this technique and its comparison with electron capture detection to determine the most suitable method of determination.

3.2.1 Optimisation of Electron Capture Detector Response

3.2.1.1 Optimisation of ECD Temperature.

Manufacturer's manuals and international intercalibration exercises (HP5890; Megginson *et al*., 1994; de Boer *et al*., 1992(b)) indicate that the operating temperature of an ECD can have an effect on the sensitivity, precision and working life of the detector . Tests were carried out to optimise the detector temperature of the Shimadzu 14A.

To simplify method development optimisation tests were undertaken using the following limited range of the compounds: HCBD, HCB, γ HCH, dieldrin, pp DDT, PCB 180 cis and trans permethrin. These compounds were selected as representative of the range of volatilities, polarities and chemical classes of analytes which would be encountered when analysing the complete range of analytes in the multi-residue method. A standard solution of these compounds dissolved in iso-octane at a concentration of 50pg/ul was prepared and used in all optimisation tests.

Initial general instrumental conditions were as follows:

i) Oven Programme, start at 70 °C hold 1min, 70-170 °C rising at 15 °C/min, 170-240 °C rising at 3 °C/min and 240-280 rising at 7 °C/min,

ii) Splitless injection set with splitter closing time at 1min.

Initially all chromatographic separations were achieved using a J&W DB 5 column of 25m length, film thickness 0.25µm , and internal diameter 0.25mm. Carrier gas was helium maintained at a constant pressure of 15 psi. Nitrogen at a constant pressure of 30 psi was used as the make-up gas.

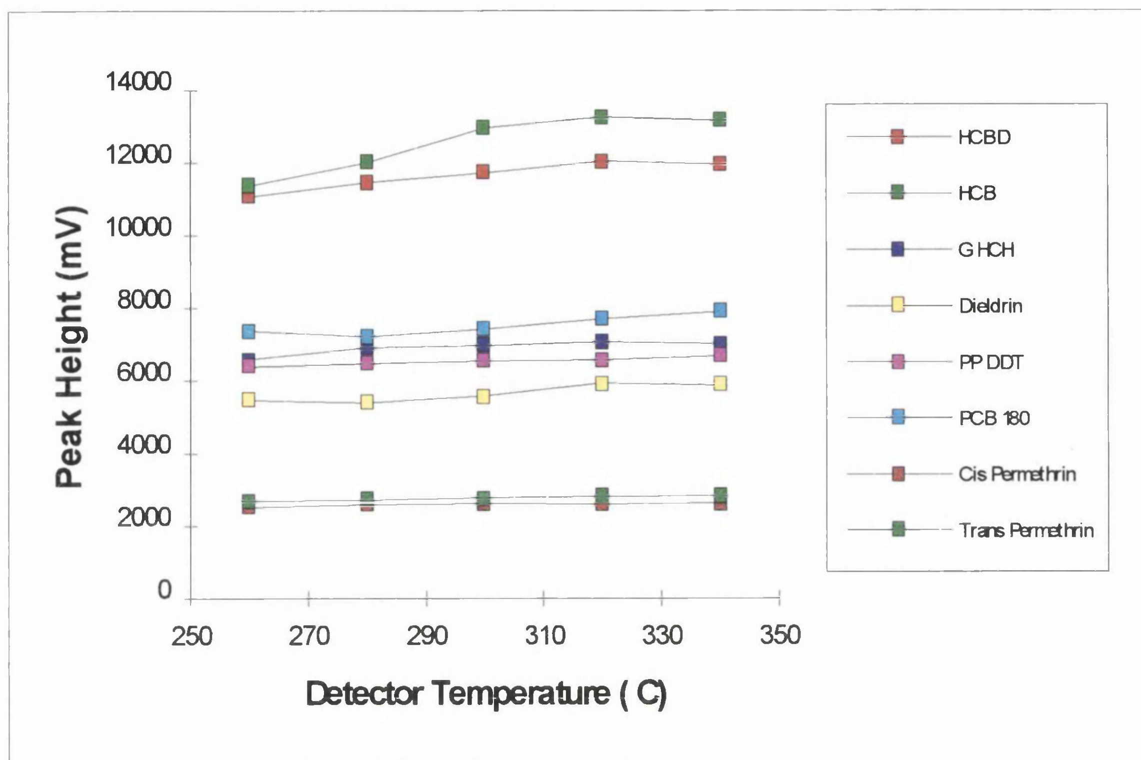
The detector response was determined for 6 replicate injections of the test standard at detector temperatures of 260, 280, 300, 320, and 340 °C. The mean peak heights and % RSD of each compound at the test detector temperatures are given in Table 4 and presented visually in Figure 1.

Table 4 Mean Peak Heights and % RSD(N=6) with Varying ECD Temperature.

Detector Temperature	260 °C		280 °C		300 °C		320 °C		340 °C	
Compound	Mean Peak Height	% RSD	Mean Peak Height	% RSD	Mean Peak Height	% RSD	Mean Peak Height	% RSD	Mean Peak Height	% RSD
HCBD	11066	4.1	11451	3.8	11720	2.9	12040	3.4	11940	2.2
HCB	11364	3.6	11995	3.2	12953	2.9	13259	3.9	13152	1.8
γ HCH	6589	3.4	6918	3.6	6966	3.8	7062	3.0	7021	2.9
Dieldrin	5460	3.3	5397	4.0	5581	4.1	5942	4.3	5873	4.2
PP DDT	6385	8.2	6475	7.8	6532	8.0	6568	8.1	6689	8.4
PCB 180	7374	2.1	7213	2.3	7426	2.1	7701	2.0	7895	2.2
Cis Permethrin	2507	4.1	2581	4.0	2620	4.1	2597	3.8	2634	3.6
Trans Permethrin	2693	4.3	2709	4.6	2771	4.0	2804	3.9	2818	3.9

The results indicate that the response of the test compounds increases with increasing detector temperature. The increase in response is greatest in the temperature range 260 -300 °C. Above 300 °C response is less sensitive to changes in detector temperature. The maximum operating temperature of the ECD cell is 350 °C. The manufacturers instructions indicate that operating the ECD cell at the maximum temperature for prolonged periods of time reduces the working life of the cell. The optimum cell temperature was selected as 320 °C. This operates the cell in a response temperature plateau, insensitive to small changes in temperature and giving maximum response.

Figure 1 Effect of Electron Capture Detector Temperature on Response



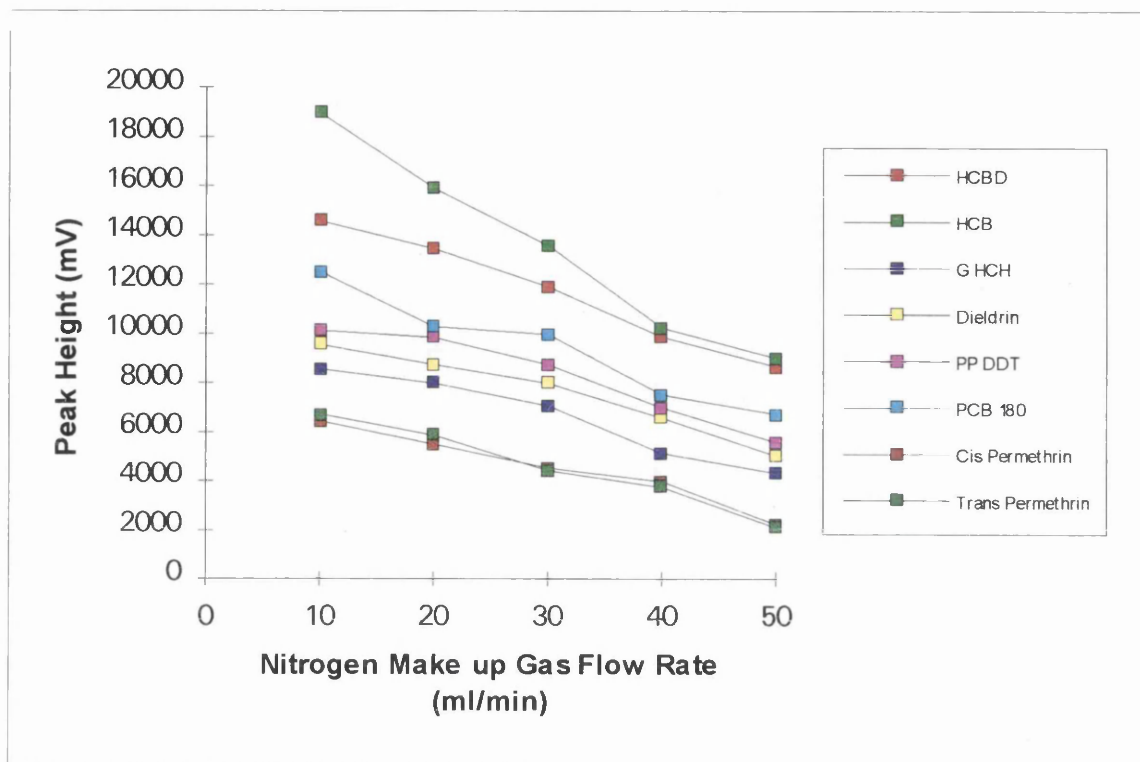
3.2.1.2 Optimisation of Nitrogen Make-Up Gas Flow Rate

The effect of flow of nitrogen make up gas at set rates between 10 and 50ml/min was tested. The detector was allowed to stabilise at each set rate for at least 30 mins. Following detector stabilisation the response of the test standard was measured for replicate injections (3) at each flow rate. The instrument conditions were as follows : Splitless injection, spliter closing 1 min, oven program 40 °C hold 1min, 40-170 °C at 15 °C/min, 170-240 at 3 °C/min, 240-280 at 7 °C/min, detector temperature 320 °C. The mean peak heights for tested gas flow rates are given in Table 5 and presented visually in Figure 2.

Table 5 Effect of Nitrogen Make-Up Gas Flow Rate on Electron Capture Detector Response

	Mean Peak Height				
Nitrogen Make-up Gas Flow Rate ml/min	10	20	30	40	50
HCBD	14568	13457	11890	9856	8657
HCB	18975	15894	13581	10235	9012
γ HCH	8563	7985	7052	5126	4325
Dieldrin	9546	8739	7981	6574	5012
PP DDT	10135	9845	8748	6987	5572
PCB 180	12498	10274	9953	7512	6719
Cis Permethrin	6423	5482	4528	3986	2218
Trans Permethrin	6721	5870	4428	3742	2085

Figure 2 Effect of Nitrogen Make up Gas Flow Rate on Electron Capture Detector Response



The results indicate that make up gas flow has a significant effect on detector response. Small variations in flow have a large effect on response. Detector response increases with decreasing flow rate. At low flow rates a high response is obtained but the chromatograms are of poor quality with a noisy baseline and significant peak tailing. At high flow rates the baseline is stable with good peak shape. Manufacturer's information recommends the use of high flow rates e.g. 40-60 ml/min to prolong detector working life and to prevent GC detector contamination.

A make up gas flow rate of 25 ml/min was selected as the optimum. This gives maximum detector response with minimum deterioration in peak shape and baseline stability.

3.2.2 Optimisation of Injection Technique

As indicated earlier (Section 2.2.1) on-column and splitless injection are the two main techniques used in multi-residue methods in environmental analysis. Both of these injection systems were available on the Shimadzu 14A instrument. A series of tests were undertaken to optimise both of these injection techniques and compare performance. The standard, GC column, and GC oven program outlined in section 3.2.1, were used for all tests. The optimised conditions of nitrogen flow rate (25ml/min) and ECD temperature (320 °C) as determined previously were used.

3.2.2.1 Optimisation of Splitless Injection

Variables tested i). glass wool packing ,ii) splitless time, iii)injector temperature.

i) Packing Material

The introduction of a small piece silanised glass wool into the injection liner to improve mixing and transfer of the sample to the column was investigated. Replicate injections (6) of the test standard with and without wool in place were carried out and the response recorded. The mean peak height with RSD for each test compounds is presented in Table 6.

Table 6 Mean Peak Height of Splitless injection With and Without Glass Wool.

Compound	Liner		Glass Wool	
	Mean Peak Height	% RSD	Mean Peak Height	% RSD
HCBD	11650	3.2	12018	2.9
HCB	12789	2.8	13056	2.7
γ HCH	7089	2.7	7008	2.8
Dieldrin	7881	2.8	7454	3.0
PP DDT	8457	4.6	7485	8.9
PCB 180	9742	2.4	9789	2.2
Cis Permethrin	4127	2.9	3741	4.5
Trans Permethrin	4560	3.1	3697	4.6

The results differ to those found by other workers (Grob & Neukom,1984). No improvement in response, peak width at half height or peak shape are observed. The peak height of PP DDT reduced sharply for the last 3 injections. These three injections resulted in the significant decrease in response and increase in RSD observed for PP DDT. It may be possible that the glass wool trapped small pieces of septa rubber or other extraneous material providing a site for the DDT to be degraded. The test injections were of "clean" standard solutions it is likely that effects due to build up of dirt or septa particles would be increased for sample extract injections.

The results indicate that the introduction of glass wool to the injection liner does not improve the analysis of OCPs by splitless injection.

ii) Splitless Time

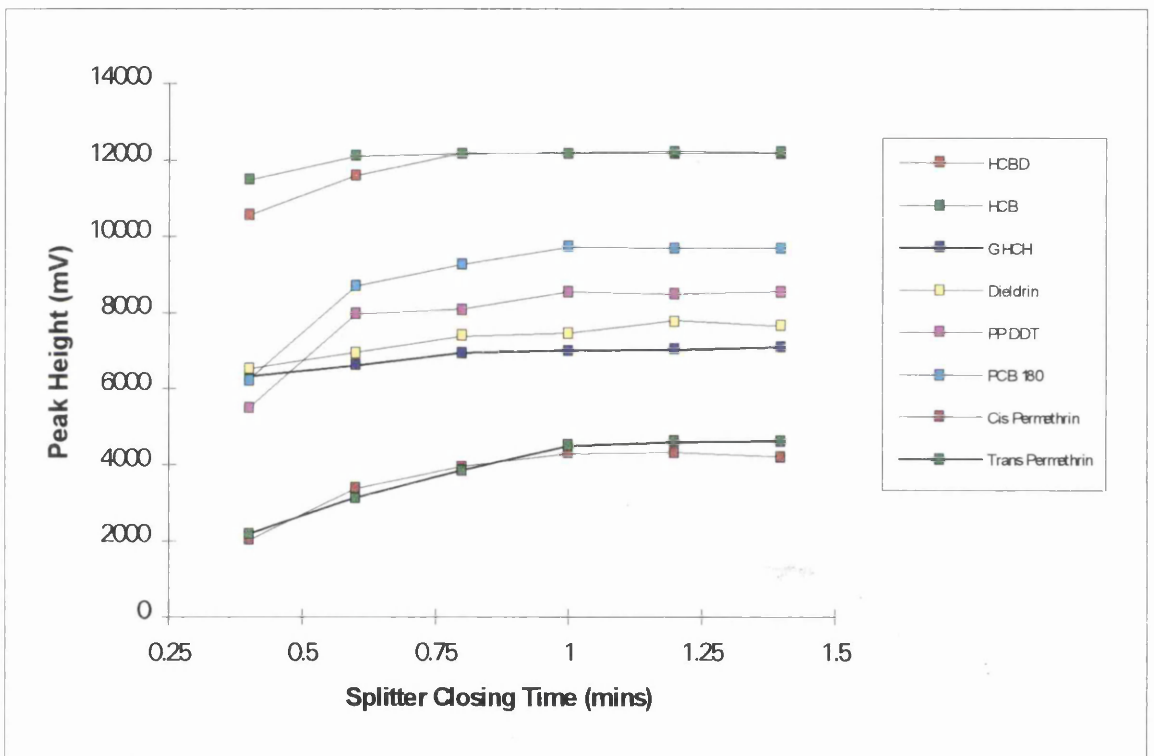
Using the instrument conditions used in test i) and the test standard solution the effect of splitter closing time on detector response was investigated.

The length of time that the splitter was closed for was varied between 0.4 and 1.4 mins in 0.2 min steps. The response for each analyte at each splitter closing time was recorded and presented in Table 7 and Figure 3.

Table 7 Effect of Spliter Closing Time on Response

	Peak Height					
Spliter Closing Time	0.4 mins	0.6 mins	0.8 mins	1 mins	1.2 mins	1.4 mins
HCBD	10560	11589	12170	12174	12179	12175
HCB	11468	12096	12158	12186	12218	12195
γ HCH	6324	6609	6946	7013	7035	7089
Dieldrin	6523	6956	7386	7458	7789	7648
PP DDT	5489	7956	8094	8542	8478	8567
PCB 180	6237	8692	9268	9715	9697	9702
Cis Permethrin	2008	3372	3945	4291	4318	4207
Trans Permethrin	2185	3126	3857	4489	4588	4621

Figure 3 Effect of Splitter Closing Time on Response



The results indicate the splitter closing time has a significant effect on response. The effect differs for each analyte. The largest effects are observed on the least volatile compounds. Increasing the splitter time from 0.4 to 1.4 min results in a 13 % increase in response for HCBD and a 53 % increase in response for trans permethrin. The length of the splitter closing time has an additional effect on the quality of the chromatogram. A splitter closing time of 0.4 min results in a high quality chromatogram with a small sharp solvent peak ,well defined analyte peaks and a stable baseline. A closing time of 1.4 min results in a poorly defined solvent peak and peak tailing for HCBD and HCB.

1 min was selected as the optimum splitter closing time producing maximum response without loss of peak shape.

iii) **Splitless Injector Temperature**

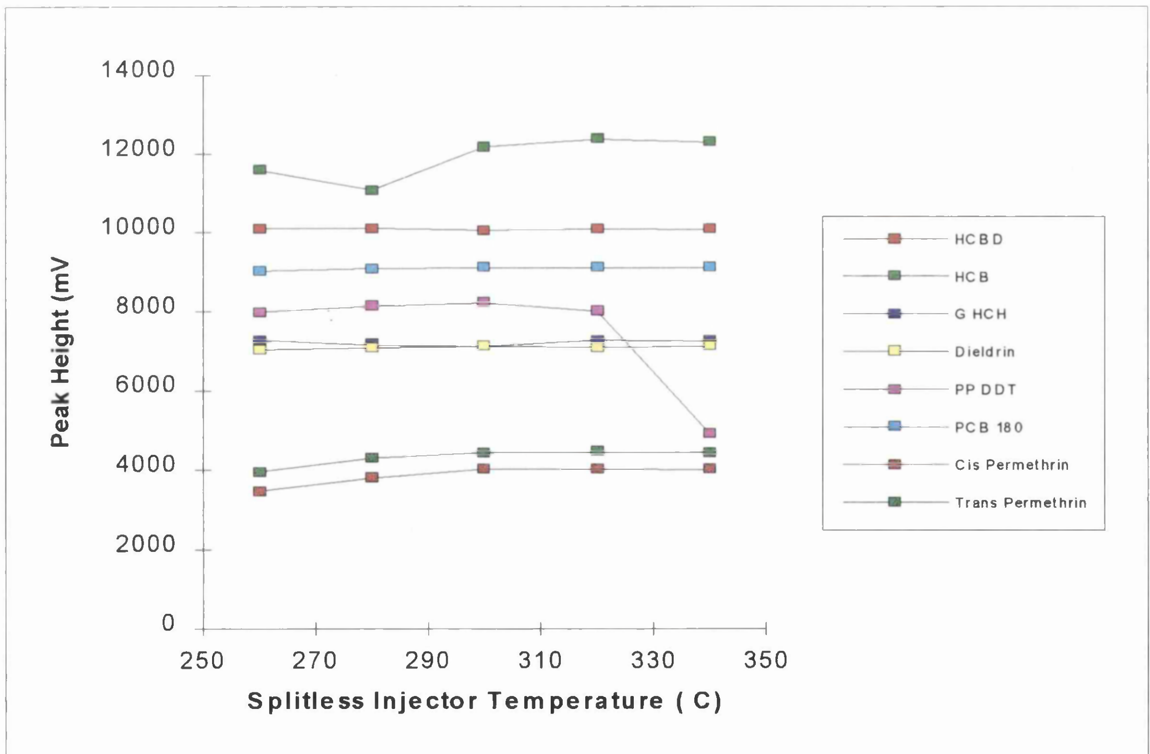
Using the instrument conditions given in test i) and the optimum settings previously determined the response for the test standard was measured over a range of injector temperatures.

The temperature of the injector was tested over the range 260 and 340 °C in 20 °C increments. The response of each analyte was recorded at each temperature and are presented in Table 8 and Figure 4.

Table 8 Effect of Injector Temperature on Response

	Peak Height				
Injector Temperature	260 °C	280 °C	300 °C	320 °C	340 °C
HCBD	10098	10102	10059	10087	10078
HCB	11580	11073	12170	12370	12282
γ HCH	7275	7148	7113	7271	7245
Dieldrin	7025	7079	7106	7096	7110
PP DDT	7985	8127	8207	8009	4892
PCB 180	9027	9091	9106	9099	9104
Cis Permethrin	3472	3798	4015	4013	4010
Trans Permethrin	3947	4301	4436	4447	4441

Figure 4 Effect of Splitless Injector Temperature on Response



The results indicate that splitless injector temperature has an effect on response. The magnitude of the effect is analyte specific. HCBD, HCB, γ HCH dieldrin and PCB 180 are not sensitive to changes in splitless injector temperature over the test range. Cis and trans permethrin show a 15% increase in response as the temperature increases from 260 to 300 °C with no further increase in response in the temperature range 300 to 340 °C . The response of PP DDT does not change significantly in the temperature range 260 to 300 °C, however, in the range 300 to 340 °C the response decreases by approximately 40 %. This dramatic decrease may be due to thermal degradation of DDT to DDD and DDE.

300 °C was chosen as the optimum splitless temperature. This gives maximum response for all analytes with minimal degradation of PP DDT.

3.2.2.2 Optimisation of On Column Injection.

To optimise the on-column injection two instrument parameters were tested i) variation of Injector/Column Oven initial temperature and ii) variation of the injector temperature programme/column oven isothermal hold time.

The GC oven, column and detector conditions were as described in section 3.2.1. A 1m deactivated 0.53 mm internal diameter uncoated fused silica retention gap was coupled before the analytical column. All injections were made using an autosampler onto the retention gap. The initial injector program was 70 °C hold 1 min, 70-280 °C at 100 °C/min hold at 280 °C for remainder of program.

i) Optimisation of Injector / Column Oven Initial Temperature.

As discussed previously the optimum on-column injection should introduce as a liquid the sample plus solvent into the retention gap. Manufacturers suggest that at injection the injector and column oven temperatures should be the same. This temperature should be 5-20 °C below the boiling point of the sample solvent .

Iso octane, boiling point 99.24°C was the sample solvent. The injector/oven initial temperature was tested between 60 and 100 °C in 10 °C steps with injections of the test standard solution. The response of each analyte was recorded for each temperature and the results presented in Table 9.

Table 9 Effect of On Column Injector / Column Oven Initial Temperature on Response.

	Peak Height				
Injector/Oven Temperature	60 °C	70 °C	80 °C	90 °C	100 °C
HCBD	10080	10102	10059	9857	8452
HCB	11581	11073	12170	11470	10158
γ HCH	7148	7139	7107	7057	5945
Dieldrin	7045	7079	7046	7096	6589
PP DDT	8076	8137	8077	8009	7987
PCB 180	9075	9091	9106	9098	9058
Cis Permethrin	3842	3798	4015	4003	3997
Trans Permethrin	4108	4301	4436	4308	4315

The results indicate that initial injector/oven temperature has an effect on response. This effect is greatest on volatile compounds such as HCBD. The response for volatile compounds decreases as the injector / oven initial temperature approaches the boiling point of the solvent. This may be caused by rapid vaporisation of the solvent in the retention gap forcing some of the analytes back out of the injector and not onto the column.

70 °C was chosen as optimum initial temperature. This temperature is low enough to prevent loss of analytes but not so low as to significantly increase the analysis time.

ii) Optimisation of the Injector Program / Column Oven Initial Isothermal period.

As previously discussed the optimum injection condition is to slowly evaporate the solvent, leaving the analytes on the retention gap. The injector is then ballistically heated, removing the analytes from the retention gap with the column oven held at a low temperature to trap the analytes in a narrow band at the start of the column.

The injector programmes / oven isothermal periods were varied and injections of the test standard solution undertaken. The response of each analyte was recorded for each injector programmes / oven isothermal periods and the results presented in Table 10.

Table 10 Effect of Injector / Column Oven Isothermal Period on Response.

	Peak Height							
Oven Isothermal Period	1min	1min	1min	1min	0.5 min	0.2 min	2 min	1 min
Injector Program	70 °C hold 1min, 70-280 at 100 °C/min	70 °C hold 0.5 min, 70-280 at 100 °C/min	70 °C hold 0.2 min, 70-280 at 100 °C/min	70-280 °C at 100 °C/min	70 °C hold 0.2min, 70-280 at 100 °C/min	70 °C hold 0.2 min, 70 280 at 100 °C/min	70 °C hold 0.2 min, 70 280 at 100 °C/min	70 °C hold 0.2 min, 70 280 at 50 °C/min
HCBD	10507	10389	10604	9875	10435	10402	10596	10572
HCB	12368	11958	11428	10680	12894	13057	11875	11578
γ HCH	7193	7257	7058	6675	7389	7014	6857	7354
Dieldrin	7834	7891	8025	8014	8094	8075	8125	7956
PP DDT	9257	8247	8852	8478	7658	7985	8325	8756
PCB 180	9327	9851	9273	9621	9496	9526	9376	9641
Cis Permethrin	3854	3985	3598	4095	4002	4210	4215	4091
Trans Permethrin	4521	4307	4850	4325	4317	4608	4652	4483

The results indicate that the injector program and oven isothermal period has a very limited effect on analyte response. The largest effects are on the most volatile compounds. HCBD and HCB demonstrate slightly lower response using the injector program with no hold period before the ballistic heating of the injector.

An injector program of 70 °C hold for 0.2 min, 70 - 280 °C at 100 °C/min hold at 280 °C for remainder of programme, and oven isothermal period of 1min were chosen as the optimum conditions

3.2.2.2 Comparison of On-Column and Splitless Injection Techniques

The optimised splitless and on-column injection conditions were used to compare the performance of these two injection techniques. Six replicate injections of the test standard were performed on each injector the mean response (MR) and RSD are listed in Table 11.

Table 11 Comparison of On-Column and Splitless Injection Techniques

	Injection Technique			
Compound	Splitless		On-Column	
	Mean Peak Height	%RSD	Mean Peak Height	%RSD
HCBD	11450	1.5	12385	1.6
HCB	12574	2.2	12476	2.1
γ HCH	7283	2.9	7424	2.8
Dieldrin	7582	3.4	8410	3.7
PP DDT	8372	6.3	8652	3.4
PCB 180	9573	4.2	9428	3.0
Cis Permethrin	4078	4.7	4108	4.1
Trans Permethrin	4420	4.6	4609	4.2

The response and quality of chromatograms for each type of injection system is similar and selection of a best technique judged by these criteria alone is not valid.

In practical terms the on-column injector is simpler to set up and use. The splitless injector relies on a clean deactivated liner to avoid decomposition of thermally labile compounds. In routine use considerable effort is required to maintain a clean liner. The stated requirement is the need for a robust routine analysis method, on-column injection is easier to set up and maintain and is therefore selected as the optimum injection technique.

3.2.3 Optimisation of Gas Chromatographic Separation

3.2.3.1 Introduction

Determination optimisation tests were undertaken with "clean" solutions of standards containing no matrix interference's such as co-eluting compounds. Sample extracts of samples can contain a variety of pesticides and interfering compounds. To minimise possible errors at the detection step by co-eluting compounds the separation of compounds must be maximised.

The class of compounds known as the PCB's contains 209 individual chlorinated biphenyl isomers spanning 10 homologous series (1-10 chlorine atoms per biphenyl). Commercial mixtures (e.g. Aroclors 1221, 1016, 1242, 1254, and 1260; Clophens A30, A40, A50, and A60) have been shown to contain at least 132 congeners (Schulz *et al.*,, 1989). GC analysis of environmental samples containing PCBs produce complex chromatograms containing large number of peaks. Within the complex pattern of PCB peaks other compounds such as organochlorine and pyretheroid pesticides may be hidden or enhanced. Present column technology does not have a single capillary GC column with the ability to routinely separate all the PCB congeners from organochlorine pesticides (Larsen, 1995). To minimise false positive results and interference's due to co-elution it is normal practice to separate environmental sample extracts into two fractions prior to GC analysis. Literature studies indicate that Fraction 1 usually contains the PCBs, and some non polar organochlorine compounds such as HCBD, HCB, DDE, and aldrin. Fraction 2 usually contains the more polar organochlorine and pyrethroid pesticides such as HCH, dieldrin, endrin, endosulfan, DDD, DDT, and permethrin. Optimal conditions for this fractional separation of sample extracts was determined in section 3.4.1.

To eliminate the need to alter the GC conditions for the different fractions the GC programme was investigated with a view to attaining an acceptable common set of GC conditions.

Gas chromatographic separations are based upon the partition of compounds between two phases during a continuous , dynamic operation driven by a carrier gas flow. One of the phases is *stationary*, the other is *mobile.* In gas chromatography the carrier gas flows through the separation column as the mobile phase and is the medium for the transportation of the analytes. The stationary phase is a thin liquid film with which the analytes temporarily interact. The mass transport of analytes takes place in the mobile phase (carrier gas). An analyte that is mainly found in the mobile phase during the separation process will have a short retention time. Chromatographic separation takes place in the stationary phase with analytes undergoing weak or strong interactions with the stationary phase. To obtain good separation the mixture of analytes must exhibit a variety of interactions with the stationary phase.

The factors which effect separation are :

a) stationary phase interactive characteristics
b) column physical dimensions e.g. length, internal diameter, and film thickness
c) temperature programme
d) carrier gas type and velocity

Financial constraints on the study restricted the purchase of a wide variety of columns to investigate the effects of different stationary phase and column dimensions on analyte separation efficiency.
Results from international inter laboratory exercises suggest that medium polarity phases such as DB 5 (5% -phenyl methyl polysiloxane) or SE-54 (5% diphenyl, 1% vinyl dimethyl polysiloxane) provide the best separation of a broad range of organochlorine contaminants (de Boer *et al.*, 1992(a)). These exercises also recommend that the column internal diameter should not exceed 0.25 mm, the column length should not be shorter than 50 m and that the film thickness should be in the range 0.1 - 1 µm.

The column used in prior tests to optimise the detector and injector conditions did not meet the above criteria. To meet the recommendations the following column was purchased for the GC analysis.

Column Manufacturer : J & W .
Stationary Phase : DB - 5
Length : 60 m
Internal Diameter : 0.25mm
Film Thickness : 0.25µm

During these optimisation investigations only helium and nitrogen were available as carrier gases. Standard texts (Schomburg, 1990) indicate that nitrogen is unsuitable for use as a carrier gas in temperature programmed capillary GC analysis. Helium was therefore used as the carrier gas . As a result of this the only two variables available for optimisation of analyte separation were carrier gas velocity and column temperature controlled by the oven temperature.

3.2.3.2 Optimisation of Carrier Gas Linear Velocity

For capillary columns, it is easier and more convenient to consider carrier gas in terms of average linear velocity (speed of the carrier gas in cm/sec) rather than flow rate (volume of the carrier gas in ml/min). Column efficiency is dependent on the average linear velocity of the carrier gas since the carrier gas flow and not the volume influences the speed of the mass transport through the column. At low gas velocities peak broadening and a loss in resolution and sensitivity occurs as a result of diffusion of the analytes in the direction of the column axis. At high carrier gas velocities peak broadening and a loss in resolution occur as a result of slow mass transfer of the analytes between the phases. i.e. the time required for the "evaporation" of the analytes from the stationary phase is a significant portion of the time required for the analyte to reach the detector. Thus for maximum separation the optimum carrier gas velocity is likely to be some mid range velocity.

Average linear velocity is calculated by injecting a non retained compound and determining its retention time. The average linear velocity is calculated as follows:

$$u = L / t_r$$

where: u = average linear velocity (cm/sec)
L = column length (cm)
t_r = retention time of a non-retained peak (sec)

As oven temperature increases the carrier gas viscosity increases and column velocities decrease, therefore, for a given analysis carrier gas velocities must be determined at a consistent temperature.

200 °C was selected as the most appropriate temperature to measure the carrier gas velocity. Using an ECD as a detector the carrier gas velocity was measured by placing a small drop of Dichloromethane in an autosampler vial and allowing the DCM to evaporate. The GC vial was then sealed and a portion of "air" from the vial injected to the chromatographic system.
Standard published van Deemter plots (Schomburg,1990) indicate that for a 0.25µm film thickness the optimum Helium gas velocity is 25cm/sec. Using the technique described above the Shimadzu 14A column head pressure was increased to obtain a carrier gas velocity of 25 cm/sec.

3.2.3.3 Optimisation Oven Temperature Program

Column temperature has a significant effect on the retention times of sample components (Schomburg,1990) and in practice is the parameter which chromatographers most frequently alter to achieve the desired compound separation. As the temperature rises the interactions between the analyte and the stationary phase can vary (usually diminish). In addition with increasing temperature the vapour pressure of an analyte increases logarithmically. The higher the partial pressure of the analyte within the chromatographic system the faster it will migrate through the column. In temperature programmed GC analysis, analyte vapours spend the majority of the residence time at the start of the column. Only when the temperature rises to within a few degrees of the retention temperature do they begin to move appreciably through the column. Analyte vapour release at the retention temperature may be approximately described as evaporating out of the stationary phase in the same way that vapours in a distillation column boil out from a solution mixture at their boiling point. The major difference is that in GC analysis the retention temperature for vapours are far below their boiling point. For the separation of mixtures of compounds which belong to similar chemical classes, and exhibit very similar chemical properties and polarities this is the major method of separation.

A Fraction 1 and a Fraction 2 standard was prepared in iso -octane. The fraction 1 standard contained the compounds HCBD, HCB, PCB 28, PCB 53, PCB 52, aldrin, THE, OP DDE, PCB 101, PCB 112, PP DDE, PCB 118, PCB 153, PCB 105, PCB 138, PCB 156, PCB 180, and DCBP at approximately 40ug/l. The Fraction 2 standard contained the compounds α HCH, β HCH, γ HCH, THE, Endo A, dieldrin, endrin, Endo B, PP DDD, OP DDT, PP DDT, cis permethrin, trans permethrin, and DCBP at approximately 40 ug/l. In addition to the above standards test solutions of 1ml of iso octane were prepared spiked with only 2 or 3 compounds of very different volatilities e.g. α HCH, dieldrin and cis permethrin. These solutions were used to unambiguously identify closely eluting compounds in situations where their identity was not clear.

The Fraction 1 &2 standards were analysed with a test series of oven programmes.

The criteria used to evaluate the optimum separation were ; (a) resolution of op DDE and PCB 101, (b) resolution of cis and trans permethrin, (c) general separation performance and (d) time required for analysis.

The resolution of two analytes is large if the average width of the peaks is small relative to their retention difference. The larger the resolution the more efficient the separation process. This may be expressed numerically as follows:

$$R = t_R / b$$

where : R is the resolution,

b is the average width of peaks,

t the retention time in seconds.

In practice this is more frequently and easily calculated using the following formula :

$$R = 2*\{(t_{Ra} - t_{Rb}) / (b_{0.5a} + b_{0.5b})\}$$

where : R is the resolution,

t_{Ra} is the retention time in seconds of analyte a,

t_{Rb} is the retention time in seconds of analyte b,

$b_{0.5a}$ is the peak width at half height in seconds of analyte a,

$b_{0.5b}$ is the peak width at half height in seconds of analyte b.

The series of oven programmes were investigated, retention times of analytes, resolution of critical separation and total analysis times were used to evaluate the oven temperature programs. The program listed below in Table 12 was selected as the optimum oven temperature program. Examples of the separation of fraction 1 and fraction 2 target analytes achieved using this program are shown in Appendices 5 and 6.

Table 12 Optimum Gas Chromatograph Oven Temperature Program

Initial Temperature	70 °C hold for 1 min
1st Ramp	70-180 °C at 20 °C/min Hold at 180 °C for 2 min
2 nd Ramp	180-230 °C at 1.2 °C/min Hold at 230 °C for 10 min
3rd Ramp	230-280 °C at 3.5 °C/min Hold 15 min.

All the gas chromatograph optimisation work described previously was undertaken in the early stages of the study. As the study progressed new analytical instrumentation became available for use. This included a HP5890 Gas Chromatograph fitted with an HP7673 autosampler, on-column and splitless injectors and dual Electron Capture detectors.

3.2.3.3 Conclusions

Parameters (ECD make-up gas flow rate and injection techniqu) which had been identified as critical in the Shimadzu GC performance were optimised by the method applied to the Shimadzu and described earlier. A major advantage of the HP5890 GC was the availability of electronic pneumatically controlled carrier gases. This system allows carrier gas flows to be accurately set from the GC control pad. While not improving the chromatography this system made initial setting up of the instrument and routine maintenance much easier. The optimised parameters for the HP5890 GC are listed in Table 13.

Table 13 Optimised Parameters for HP5890 Gas Chromatograph

Instrument	HP 5890 Series II Gas Chromatograph fitted with an HP 7673 Autosampler
Injection technique	On column
Injection Volume	1µl
Injector Program	70 °C hold for 0.2 min 70 - 300 °C at 100 °C/min Hold at 300 °C for 45 mins
Carrier Gas	Helium Average linear velocity at 200 °C 25cm/sec
Retention Gap	Deactivated fused silica, Length 1M, ID 0.53mm.
Analytical Column	J&W DB - 5, Length 60M, ID 0.25mm, Film thickness 0.25µm
Column Oven Program	70 °C for 1 min, 70 - 180 at 20 °C/min, Hold at 180 °C for 2min, 180 °C - 230 °C at 1.2 °C/min, 230-280 °C at 3.5 °C/min Hold at 280 °C for 15 min.
Detector	Nickel 63, Electron Capture
Detector Temperature	320 °C
Make Gas	Nitrogen 30mls/min

As the HP 5890 GC became available for use a more advanced data collection integration system was made available for use. This new system was a UNIX based VG Data Systems XCHROM chromatography data acquisition system. While not having a direct impact on the analytical methodology this system offered improved peak integration and more sophisticated data handling routines. The enhanced data system significantly reduced the time required to process chromatograms.

Both the Shimadzu 14A and the HP5890 system described were used for the subsequent sample preparation method development work.

In the latter stages of the study a Peak Scientific hydrogen gas generator became available for use. This enabled Hydrogen to be used as a carrier gas. It was anticipated that the change from Helium to Hydrogen as a carrier gas which could be used at a higher velocity would result in improved resolution of closely eluting compounds. It was hoped that these sharper narrower peaks would improve sensitivity and reduce GC analysis time.

Initial trials using Hydrogen as a carrier gas were encouraging on the Shimadzu 14A and disappointing on the HP5890. Resolution of closely eluting peaks is improved on both instruments but the sensitivity of the HP5890 detector is reduced 5 fold (No data presented).

Manufacturer's manuals and standard chromatography texts offer no explanation for this large decrease in sensitivity. Initially the HP manufacturer was unable to offer advice on this drop in sensitivity or a solution to the problem. Further discussion with the manufacturer and other users of HP gas chromatographs revealed that the potentiometer setting of the Electron Capture Detector could be altered by means of a variable potentiometer on the detector control board. Past experience with the Shimadzu 14 A GC which had a variable detector potentiometer had indicated that increasing the potentiometer setting results in an increase in detector signal. This increase in detector signal is also reflected in an increase in " background noise ". It was not clear what overall effect this would have on the sensitivity of the detector.

Following alteration of the potentiometer setting, tests showed a dramatic improvement in detector sensitivity. This increased the response of electron capturing compounds while only causing a minimal increase in background detector noise. Fine adjustments of the potentiometer setting resulted in an overall 10 fold increase in sensitivity.

Standard Van Deemter curves indicate that the optimum carrier gas velocity for hydrogen is 40 cm/sec. The column head pressure on both the Shimadzu 14A and HP5890 were altered to set the carrier gas velocity at 200 °C to 40 cm/sec.

Comparison of the retention times and resolution of critical analytes using helium and hydrogen as carrier gases confirmed the superior performance of hydrogen as a carrier gas (No data presented).

Standard chromatography texts indicate that reducing the internal diameter of the capillary column has a significant effect on resolution. In addition to the financial constraints of purchasing a variety of columns a further consideration in the use of narrow bore columns had been the high gas pressures which were required when using helium as a carrier gas. These higher pressures cause a greater incidence of column leaks requiring more system maintenance. Columns from the same manufacturer with identical physical dimensions and stationary phase do not always exhibit the same performance. Chromatographers have become aware that occasionally some columns have exceptional performance for their dimension and stationary phase. Manufacturers have become aware of the commercial value of these "super" columns and following identification of these columns from quality control sell them for a premium. Towards the latter stages of this study a "super" column of the type given overleaf was purchased and optimised as described previously.

Column Manufacturer : HP
Stationary Phase : Ultra -2
Internal Diameter : 0.2mm
Film Thickness : 0.33µm
Length : 50M

All previous tests had failed to separate the Fraction 1 analytes from the Fraction 2 analytes. Tests were carried out using the new HP Ultra 2 column with hydrogen as a carrier gas the chromatogram is given in Appendix 7 the tests show that separation of all components of F1 and F2 is achieved.

This successful GC separation of F1 and F2 analytes allows a possible step removal in the extract clean-up resulting in a significant saving in analysis time. Extracts are GC screened without prior fractionation. Extracts in which none of the nine target PCB congeners are detected receive no fractionation into F1 and F2 splits. The results for other compounds are accepted and no further analysis is required. This decision is supported by the reasoning that PCBs are present in the environment as a complex mixtures of up to 150 congeners. These mixtures are well categorised and if the sample is contaminated with PCBs one or more of the 9 target PCBs would be detected. Should target PCBs be detected the sample extract would require to be separated and both fractions GC analysed to allow accurate identification and quantification. This screening was extremely useful for river and waste water samples where PCBs are rarely detected. Screening was not used for matrices such as sediment and biota where PCBs are detected in most samples.

3.2.4 Calibration

In chemical analysis the detecting instruments are routinely calibrated by analysing a series of at least three or four standards in which the concentration of the analyte is known. These calibration standards are measured in the analytical instrument under the same conditions as used for the test samples. The instrument response for the standards is used to construct a calibration graph and this graph is used to determine the concentration of the unknown samples. It is convention for the instrument response to be plotted on the *y axis* and the concentration on the *x axis.*

Historical evidence suggests that in early environmental organic analysis calibration was a major source of error. Intercomparison exercises (Tuinstra et al., 1989, Wells *et al.*, 1988; Wells *et al.*, 1989*(a))* suggest that the main sources of errors in the determination of organochlorine compounds in environmental matrices occur during; (1) the preparation, storage and handling of calibration solutions, and (2) during the calibration of the gas chromatograph. To eliminate and reduce possible errors of these types preparation of standards and GC calibration have been studied in detail.

3.2.4.1 Handling Organic Solvents.

In traditional aqueous based analytical chemistry the ability to transfer and dispense accurately different volumes is taken for granted. Glassware (standard flasks, glass pipettes, and pipettemen) found in analytical laboratories is designed to measure aqueous solutions, and as such are not necessarily suited for use with organic solvents.
A series of simple tests were undertaken to evaluate the suitability of conventional air displacement pipettemen, positive displacement pipettemen, and glass microsyringes as methods of dispensing liquids.

Air displacement pipettes are the most commonly used type of automatic pipette. The liquid sample is displaced by an air interface. These pipettes are recommended by the manufacturers for all pipetting needs involving fluids of moderate viscosity and density. The manufacturers specifications indicate that accuracy of ±0.5% and a precision of ±0.4% can be achieved using these pipettes.

Positive displacement pipettes rely on the liquid being displaced by the piston. The liquid is in direct contact with the piston. They are ideally suited to the pipetting of problem fluids with high viscosity, volatility or density. The manufacturers specifications indicate that accuracy of ±1.0% and a precision of ±0.8% can be achieved using these pipettes.

The microsyringes used in this test were precision glass bodied syringes with a teflon coated plunger, and precision machined teflon tip. The manufacturers specifications indicate that an accuracy and precision of ±1.0% can be achieved using this syringe.

10, 50, 100, 500, and 1000 µl volumes of water, iso-octane and acetone were repeatedly (n=15) dispensed into a sealable weighing jar and the weights recorded. For water a correction factor accounting for local variations in temperature and air pressure was applied to these weights. Table 14 lists the corrected volumes delivered by each method along with the % RSD and mean % bias. Correction factors for acetone and iso octane were not available and the actual weight of solvent delivered is listed in tables 15 and 16.

The volume of water dispensed was determined at temperature (T) from the mass (M) using the following equation:

$$V=M*Z$$

where: M - is the mean mass of the liquid delivered

Z - is the conversion factor incorporating the density of water when buoyed in air, at the test temperature and pressure.

When conducting this test the air temperature in the laboratory was recorded as 20.5 °C, no means of accurately recording the air pressure was available, therefore, the air pressure was estimated as 907 hPa (mbar) respectively, this gives a correction factor of Z = 1.0028.

Table 14 Volume of Water Delivered using Different Dispensing Methods

	10			50			100			500			1000		
	Mean Volume (mls)	Bias %	% RSD	Mean Volume (mls)	Bias %	% RSD	Mean Volume (mls)	Bias %	% RSD	Mean Volume (mls)	Bias %	% RSD	Mean Volume (mls)	Bias %	% RSD
Air Displacement	0.01006	0.6	0.72	0.05038	0.76	0.41	0.10038	0.38	0.36	0.49936	-0.13	0.21	0.99902	-0.09	0.17
Positive Displacement	0.00994	-0.6	0.78	0.05028	0.56	0.72	0.10029	0.29	0.48	0.50027	0.05	0.38	1.00215	0.22	0.15
Microsyringe	0.01011	1.1	2.29	0.05065	1.3	2.01	0.10057	0.57	1.85	0.50183	0.6	0.87	1.00843	0.84	0.75

Table 15 Mass of Iso Octane Delivered using Different Dispensing Methods

	10			50			100			500			1000		
	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD
Air Displacemen	0.00604	-12.7	8.87	0.03073	-11.2	9.12	0.05826	-15.8	8.47	0.32002	-1.7	2.82	0.66425	-4.01	1.86
Positive Displacemen	0.00684	-1.16	2.15	0.03257	-5.87	3.14	0.06853	-0.97	1.21	0.34289	-0.90	1.81	0.68957	-0.35	1.06
Microsyringe	0.0698	1.16	2.16	0.03402	-1.68	2.03	0.06946	0.38	1.54	0.34771	0.41	0.78	0.69017	-0.26	0.86

Table 16 Mass of Acetone Delivered using Different Dispensing Methods

	10			50			100			500			1000		
	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD	Mean Weight (g)	Bias %	% RSD
Air Displacemen	0.00701	-10.8	12.5	0.02957	-24.7	13.0	0.07426	-5.47	4.97	0.37510	-4.51	3.19	0.7795	-0.78	4.52
Positive Displacemen	0.00752	-4.28	5.28	0.03675	-6.44	4.78	0.07489	-4.67	3.26	0.38261	-2.59	2.87	0.7623	2.97	3.01
Microsyringe	0.00789	0.44	1.54	0.3899	-0.74	1.39	0.07877	0.27	0.80	0.39530	0.64	0.67	0.7808	-0.61	0.82

Using water for comparison purposes the air displacement pipette is the most accurate, precise and easiest to use. The air displacement pipettes, however, failed to meet the manufacturer's performance specifications for dispensing 10 and 50 μl volumes. The failure to meet the accuracy specification may in part have been caused by the estimation of the air pressure. The air pressure has no effect on the precision and the results highlight the need for careful maintenance and operation of simple but key pieces of equipment.

The positive displacement pipettes perform almost as well as the air displacement pipettes. The manufacturer's performance specifications are met for all volumes dispensed.
The microsyringes are slow and cumbersome to use in comparison with the other pipette types. The manufacturers performance specifications are narrowly exceeded for the 10, 50 and 100 μl volumes. Great care and patience was required to remove air bubbles from the syringes when dispensing small volumes. This difficulty may have contributed to the failure to meet the manufacturers performance specifications.

When using iso-octane and acetone as the test solutions the air displacement pipette is extremely difficult to use. The solution ran out from the pipette tip prior to the solution being dispensed. This loss is reflected in the results with a high negative bias and poor reproducibility for all dispensed volumes. This type of pipette is not suitable for dispensing organic solvents accurately or precisely.

The performance of positive displacement pipettes deteriorate significantly when used for organic solutions. Similar to the use of air displacement pipettes problems were experienced with solvent dripping from the pipette tip prior to delivery. This pipette was found to be unsuitable for the routine dispensing of solvents.

The performance of the microsyringe with iso-octane and acetone was similar to its performance with water. This pipette is suitable for dispensing a variety of solvents with an accuracy and precision of ±1.0% but it was concluded that it was too slow and cumbersome for routine use. The major disadvantage of the microsyringe method was the difficulty in removing small bubbles of air from the syringe barrel.

As a result of the above tests it became clear that the simplest method of dispensing known amounts of organic solution accurately and precisely was to use the microsyringe to dispense an approximate estimate of the required volume and to weigh the amount delivered. Little or no time was spent removing air bubbles. The precise amount delivered was accurately calculated from the weight. This method was subsequently used in the preparation of all standard solutions.

Laboratory standard flasks are designed for use with water at either 20 or 25 °C. Organic solvents such as acetone and iso octane have lower thermal expansion coefficients than water. As a result small changes in ambient room temperature have a greater effect on the volume of solvent standard flasks contain. This effect may be demonstrated by placing two identical standard flasks filled to the mark, one with acetone and one with water at room temperature (20°C) in a refrigerator at 6°C. On removal from the refrigerator after a two hours the flask containing acetone appears only two thirds full in contrast the water flask appears to have decreased only slightly in volume.

The day to day variation in air temperature within a laboratory (14-26 °C as observed during 1993) may cause significant errors in the preparation of standards in organic solutions when volumetric flasks are used as the volume measuring standard.

The previous dispensing solution tests demonstrate that to obtain the highest accuracy and precision standards must be prepared using solution weight to determine the amount of dissolving solvent (i.e. a weight of solvent added not final volume).

In addition to providing greater accuracy and precision, preparing standards by mass offers the following advantages: mass is simple to measure, traceable to a single balance, and can be back checked . The need for volumetric flasks is eliminated and other dissolving vessels may be used for standard preparation. For this study amber borosilicate glass bottles fitted with teflon lined screw caps were used for the preparation and storage of all standard solutions.

3.2.4.2 Preparation of Standard Solutions

Primary Standards

In all analytical methods the primary calibrant material is the foundation of subsequent measurements. For this study individual pure solid compounds were purchased from the Bureau of Community Reference Materials, the Laboratory of the Government Chemist and Promochem. These solid compounds were of certified purity which was required to be greater than 95 % purity to be acceptable. The cost of these compounds was very high (£2-300 for <10mg per individual congener) and therefore only small quantities, (generally<10mg) were purchased.

The handling of small amounts of these highly toxic individual compounds during standard preparation placed difficult constraints on the preparation. Weighing by difference (i.e. weighing the solid plus its container transferring a portion of the solid to the standard bottle and re-weighing the container) is the accepted method of weighing for primary standard preparation. Because of the small amounts to be weighed it was necessary to use a balance accurate to five decimal places i.e. 10µg. This method was found to be unsuitable for the preparation of the selected standards as the electrostatic nature of the solids made it difficult to transfer these solids without particles "flying" or sticking to surfaces. The simplest and most convenient method of standard preparation was found to be direct weighing using small hand made aluminium boats.

Iso-octane was chosen as the solvent for the preparation of all standard solutions because it was readily available in high purity (100 fold concentration gave no peaks when analysed by GC/ECD) and had a high boiling point reducing errors associated with evaporation.

The procedure for the preparation of individual primary standards is given below.

1. A small approximately $5\ cm^2$ piece of aluminium foil was roughly fashioned by hand into a small boat (the boat must be narrow enough to pass easily through the neck of an amber standard bottle).

2. The boat and a 50 ml amber bottle were thoroughly cleaned by ultrasonicating in acetone and then hexane. To avoid contamination from "finger grease" the boat was only handled using tweezers. The boat and bottle were removed from the hexane and placed in an oven at $105\ ^{\circ}c$ for 2 hours to dry.

3. The boat and bottle were removed from the oven and cooled to room temperature in a desiccator.

4. The boat was weighed accurately to 0.01mg and the weight recorded in mg (W1).

5. Approximately 5 mg of the analyte was placed in the boat. The boat plus contents were re-weighed as in step 4 and the weight recorded in mg (W2).

6. The boat plus contents were placed inside the amber bottle. The amber bottle was weighed accurately to 0.1mg and the weight recorded in g (W3)

7. Approximately 50 mls of Iso-octane were added to the amber bottle. the bottle plus solvent were weighed accurately to 0.1mg and the weight recorded in g.(W4).

8. The top was placed on the bottle and the bottle plus contents shaken thoroughly.

9. The standard concentration was calculated as follows:

$$\text{Concentration (mg/g)} = (W2 - W1) / (W4 - W3)$$

If required the concentration can be converted to mg/l by multiplying by the density of the solvent.

Independently prepared and verified commercial standard solutions of the analytes selected are where available very expensive and not available to this study. As confirmation of the preparation a second set of standards were prepared in a similar manner by a different analyst. External confirmation of standards was achieved by interlaboratory comparison and this will be discussed later.

Aliquots of both sets of standards were diluted by weight to give solutions for each analyte of equal concentration. The diluted standards were analysed by GC/ECD and the response for each analyte compared. Standards with response within 8% were accepted as having been prepared accurately. For pairs of standards with a response difference greater than 8% a third standard was prepared and analysed to determine an accurately prepared pair.

Confirmed standard solutions were labelled and the total weight of container plus standard solution recorded to 0.01g. Standard solutions were stored in a refrigerator at 6 ± 2 °C. Prior to use after storage the standard solutions were allowed to equilibrate to room temperature and then weighed. The weight of the standards were compared to the recorded weight. Differences in weight of < 0.1 g were considered acceptable. Differences in weight greater than 0.1g were thoroughly investigated. When the source of weight loss was not identified or explained new standards were prepared. Following use standard solutions were re-weighed and returned to the refrigerator. Standard solution containers tightly capped at room temperature were observed to have loose caps after a period in the refrigerator. The coefficient of expansion of glass and teflon are different and it is suggested that this leads to the teflon lined screw cap loosening. To overcome this problem standard solutions were re-tightened 2-3 hours after being placed in the refrigerator.

Intermediate and Working Solutions

Mixed Intermediate and working standards were prepared by mass using the techniques described earlier. The intermediate and working standards were stored in a refrigerator and weight controlled as per the stock standard solutions.

3.2.4.3 Calibration of the Gas Chromatograph Detector

In the previous studies the precision of replicate measurements made on the GC had been poor with an RSD as high as 8% being observed for some compounds. A recognised technique in organic analysis to improve precision and accuracy is the use of Internal or Surrogate standardisation. The terms *Internal standard* and *Surrogate Standard* are frequently interchanged by authors leading to confusion in their meaning.

In organic environmental analysis the terms are strictly defined as follows:

Internal Standard : a known amount of a compound added to the final extract immediately before the instrumental determination.

Surrogate Standard : a known amount of a compound added to the sample or sample extract at an early stage of sample preparation/extraction. This standard may be used to indicate losses of compounds during the sample preparation steps.

Throughout this study the terms are used as defined .

The key requirements for an internal standard used in gas chromatography are ;

i) that it should not be present in the sample,
ii) behave in a similar manner to the analytes,
iii) elute in a part of the chromatogram free from interference.

Numerous authors (Wells *et al*., 1985; WRC Interim Progress Report NR 2746; Hucnerfuss *et al* 1992; Abrahamsson & Ekdahl 1993; Japenga *et* al., 1987) have successfully used a wide variety of internal standards in multi-residue methods. Some of the more commonly used internal standards are listed below; dichloroalkylbenzylethers, trans heptachlor epoxide, decachlorobiphenyl, delta HCH, 2,6-dibromophenol, octachloronaphthalene or 1,2,3,4 tetrachloronaphthalene, PCBs which do not contribute significantly to the total PCB content of commercial arochlors e.g. CBs 29, 53, 112, 155 and 198.

An investigation was carried out to assess the usefulness of an internal standard. Because of ready commercial availability decachlorobiphenyl (DCBP) and trans heptachlorepoxide (THE) were

initially selected as internal standards. Both compounds were added to the test standard described in section 3.2.1 in a quantity giving an internal standard concentration of 40ug/l. The standard solution now containing internal standards was analysed six times using the on-column conditions described in section 3.2.3.3. The response for each analyte with and without internal standard correction was calculated for each injection. The mean responses with the RSD are given in Table 17.

Without Internal Standard response is given by : Response = Hght A

With Internal standard response is given by :

$$\text{Response} = \frac{\text{Hght A}}{\text{Conc A}} \quad \frac{\text{Conc I}}{\text{Hght I}}$$

Where: Hght A is the Peak Height of the Analyte

Conc A is the Concentration of the Analyte in ug/l

Hght I is the Peak Height of the Internal Standard

Conc I is the concentration of the Internal Standard ug/l

Table 17 Mean Response and RSD With and Without Internal Standards

	No Internal Standard		THE Internal Standard		DCBP Internal Standard	
	Mean	RSD	Mean	RSD	Mean	RSD
HCBD	9696	1.59	1.489	2.51	1.546	1.77
HCB	9808	3.33	1.506	2.16	1.564	1.51
γ HCH	6107	4.31	0.938	3.58	0.974	2.60
THE (ISTD)	5211	2.81	1.000	-	1.039	2.54
Dieldrin	6109	3.55	0.938	3.05	0.974	2.72
PP DDT	8116	6.92	1.246	5.29	1.294	4.54
PCB 180	8436	4.67	1.295	3.10	1.345	2.57
Cis Permethrin	4213	3.95	0.647	3.26	0.672	2.68
Trans Permethrin	4301	6.33	0.660	4.76	0.686	3.91
DCBP (ISTD)	5017	3.94	0.627	3.10	1	-
Mean		4.14		3.76		2.76

The use of THE and DCBP as internal standards improved the overall precision. The use of THE results in an a 9% reduction in RSD, and the use of DCBP resulted in a 33% reduction in the RSD. Therefore the use of internal standards for calibration was recommended.

Correct characterisation of the response curve of the ECD for each analyte is the second critical parameter in obtaining reliable analysis. It has been recommended (Wells *et al.*, 1989 (a)) that the "linear portion" of the ECD response curve should be obtained for each analyte and that samples must be diluted / concentrated so as their response lies within the "linear portion". Only responses within the linear portion can be quantified.

An experiment was conducted to determine the linear portion of the Shimadzu 14A detector.

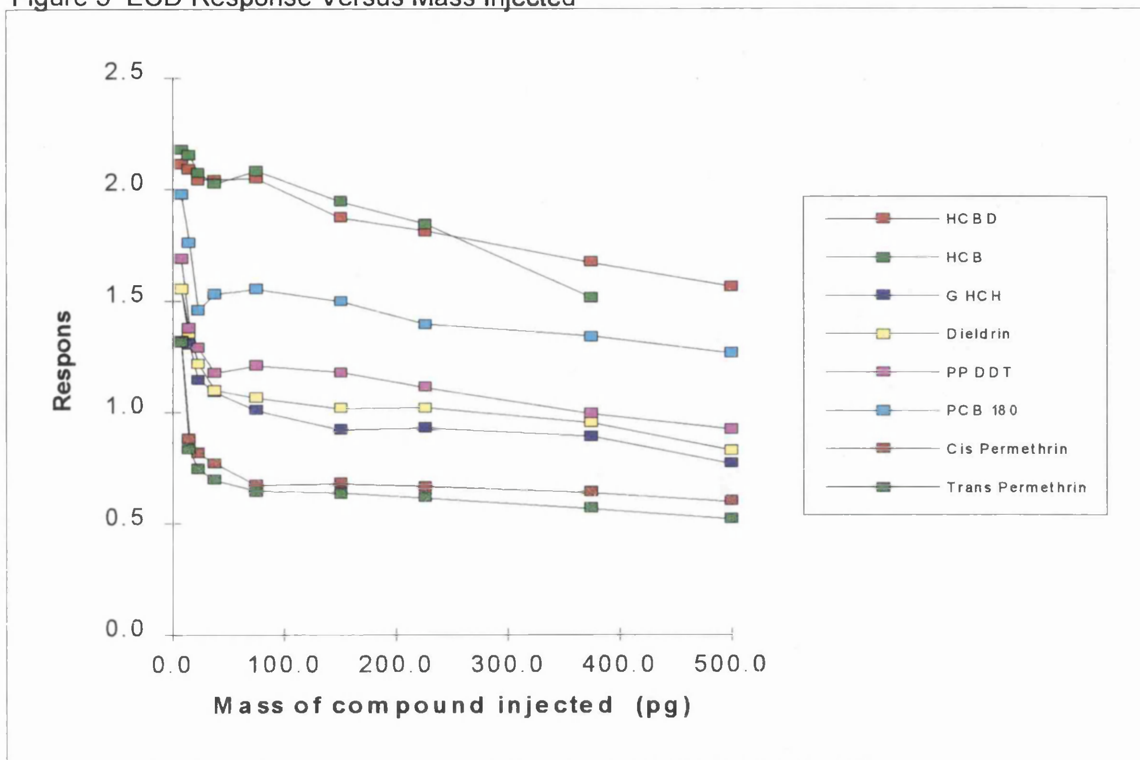
A series of standards containing each of the test analytes at the following approximate concentrations 7.5, 15, 22.5, 37.5, 75, 150, 225, 375, and 500 µg/l and containing the test internal standards at 40ug/l was analysed on the Shimadzu 14 A GC using the optimised on-column conditions. The response for each analyte was calculated using the equation given previously, the calculated responses are listed in Table 18 and presented graphically in Figure 5.

Table 18 Electron Capture Detector Response

	Mass injected (pg)								
	7.5	15	22.5	37.5	75	150	225	375	500
Compound	Response								
HCBD	2.114	2.090	2.043	2.044	2.047	1.871	1.812	1.669	1.561
HCB	2.178	2.153	2.072	2.025	2.081	1.944	1.844	1.513	*
γ HCH	1.551	1.303	1.147	1.090	1.005	0.919	0.929	0.889	0.767
THE (ISTD)	1.039	1.024	1.147	1.115	1.062	1.015	1.103	1.041	1.066
Dieldrin	1.551	1.356	1.218	1.097	1.063	1.013	1.019	0.949	0.826
PP DDT	1.690	1.373	1.287	1.176	1.207	1.180	1.109	0.991	0.923
PCB 180	1.976	1.758	1.455	1.530	1.550	1.495	1.396	1.339	1.262
Cis Permethrin	1.324	0.880	0.816	0.769	0.671	0.676	0.667	0.637	0.597
Trans Permethrin	1.310	0.830	0.742	0.695	0.644	0.636	0.613	0.566	0.519
DCBP (ISTD)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

* Peak off scale

Figure 5 ECD Response Versus Mass Injected



These results show that the linear portion of the Shimadzu 14A ECD was approximately between 7.5 - 75 pg on column for HCBD and HCB, 22.5 - 150pg for γ HCH, dieldrin, PP DDT and PCB 180 and 75 - 375 pg for cis and trans permethrin. This range is significantly above the lowest detectable concentrations . This use of only the linear portion of the detector curve would appear to be a major analytical disadvantage. In environmental analysis the challenge is to continually detect the ever lower concentrations which are found in the environment. By using only the linear portion of the ECD a high limit of detection is placed on the method. Additionally sample extracts may have to be concentrated/ diluted and GC analysed a number of times to ensure that all the analytes were analysed within their linear range. This concentration/dilution and re-analysis would increase the possibility of handling/contamination errors and significantly increase analysis time.

Against the recommendation to use only the linear portion of the detector curve it was decided that this approach was not appropriate for a multi-residue method applied to a wide variety of sample types. It was considered more suitable to use an alternative approach using a series of multilevel calibration standards to accurately describe the ECD response with each analysis batch.

From the linearity test it was clear that the ECD response varied greatly for individual compounds. The ECD was very sensitive to light highly chlorinated compounds which eluted as very sharp narrow peaks such as HCBD and HCB. In contrast the ECD was relatively insensitive to compounds with a low degree of chlorination and which due to their long retention time eluted as broader peaks. Using the chromatograms obtained from sections 3.2.2 the amount of each

compound required to produce a response approximately equal to 40pg on column of DCBP was calculated. This "equivalent response" was used to calculate the amount of each compound required to produce a series of standards which would describe the ECD response over the complete 1mV output range with the ECD set on its most sensitive attenuation. The target concentrations for the fraction 1 and 2 standards are listed in tables 19 and 20.

Table 19 Fraction 1 Calibration Standards

Compound	STDA	STDB	STDC	STDD	STDE
HCBD	0.2	1.0	4	8	20
HCB	0.5	2.5	10	20	50
PCB 28	0.5	2.5	10	20	50
PCB 53(Surr)	2.5	12.5	50	75	100
PCB 52	0.5	2.5	10	20	50
Aldrin	0.2	1.0	4	8	20
THE (INT STD)	40	40	40	40	40
op DDE	0.5	2.5	10	20	50
PCB 101	0.5	2.5	10	20	50
PCB 112 (INT STD)	40	40	40	40	40
pp DDE	0.5	2.5	10	20	50
PCB 118	0.5	2.5	10	20	50
PCB 153	0.5	2.5	10	20	50
PCB 105	0.5	2.5	10	20	50
PCB 138	0.5	2.5	10	20	50
PCB 156	0.5	2.5	10	20	50
PCB 180	0.5	2.5	10	20	50
PCB 198 (INTSTD)	40	40	40	40	40
DCBP(INT STD)	40	40	40	40	40

Table 20 Fraction 2 Calibration Standards.

	STD A	STD B	STD C	STD D	STD E
α HCH	0.2	1	4	8	20
βHCH	1.0	5	20	40	100
γ HCH	0.5	2.5	10	20	50
ε HCH (SURR)	2.5	12.5	50	75	100
THE (Int Std)	40	40	40	40	40
Endo A	0.5	2.5	10	20	50
PCB 112 (IntStd)	40	40	40	40	40
Dieldrin	0.5	2.5	10	20	50
Endrin	0.5	2.5	10	20	50
Endo B	0.5	2.5	10	20	50
pp DDD	0.5	2.5	10	20	50
op DDT	0.5	2.5	10	20	50
pp DDT	0.5	2.5	10	20	50
Bifenthrin	10	50	200	400	800
PCB 198 (INT STD)	40	40	40	40	40
Cis Permethrin	10	50	200	400	800
Trans Permethrin	10	50	200	400	800
DCBP (INTSTD)	40	40	40	40	40

Modern integrators offer a wide variety of calibration options : linear, linear point to point, quadratic, cubic etc. Tests were undertaken to determine which calibration type best suited the ECD response for each compound over the above calibration ranges.

Four additional calibration check standards with target analyte concentrations outlined in Table 21 were prepared. These additional standards were targeted specifically at the top and lower end of the normal calibration range and were approximately 10 and 90 % of the top standard. The standards and check standards were analysed sequentially in the order given below using the optimum GC conditions described previously.

Analysis sequence : A - E fraction 1GC calibration standards, 10 and 90% fraction 1 calibration check standards, A - E fraction 1 GC calibration standards, A - E Fraction 2 GC calibration standards, 10 and 90 % Fraction 2 calibration check standards, A - E fraction 2 GC calibration standards.

Table 21 Nominal Concentrations of Additional Calibration Check Standards.

	Concentration ug/l			Concentration ug/l	
Compound	10%	90%	COMPOUND	10%	90%
HCBD	2	18	α HCH	2	18
HCB	5	45	β HCH	10	90
PCB 28	5	45	γ HCH	5	45
PCB 53(Surr)	10	90	ε HCH (Surr)	10	90
PCB 52	5	45	THE	40	40
Aldrin	2	18	Endo A	5	45
THE (Int Std)	40	40	PCB 112 (IntStd)	40	40
op DDE	5	45	Dieldrin	5	45
PCB 101	5	45	Endrin	5	45
PCB 112 (Int Std)	40	40	Endo_B	5	45
pp DDE	5	45	pp DDD	5	45
PCB 118	5	45	op DDT	5	45
PCB 153	5	45	Bifenthrin	80	720
PCB 105	5	45	PCB 198 (INT STD)	40	40
PCB 138	5	45	Cis Permethrin	80	720
PCB 156	5	45	Trans Permethrin	80	720
PCB 180	5	45	DCBP (INTSTD)	40	40
PCB 198 (INTSTD)	40	40			
DCBP(INT STD)	40	40			

The ECD response for Fraction 1 and 2 GC calibration standards A - E were used to prepare a variety of calibration curves. Two types of curve were available for routine use:

Regression Curves - regression types included linear, quadratic, cubic, quadratic, quintic, and sexic.

Interpolation Curves - Interpolation types include cubic spline and point to point linear.

Linear, point to point linear, quadratic, cubic, and cubic spline calibration graphs were prepared for each analyte. Visual inspection of curves had indicated that quintic and sexic curves do not describe the data and therefore these curves were not investigated further. The internal standards were plotted as single point linear curves through the origin.

The success of these curves in describing the detector calibration was assessed by the use of descriptive calibration statistics for the regression curves and the quantification of 10 and 90% calibration check standards analysed as unknown samples.

All regression curves are of the form :

$$y= b_o + b_i x^i$$

where : i takes values between 1 (for curve type linear) to 6(for curve type sexic).

The coefficient of determination and standard error of y estimate were used as the calibration descriptive statistics.

The coefficient of determination was defined as follows:
For each data point a value of y (y_{ipred}) can be predicted from the calibration curve at the position x_i.
For each data point a residual between the actual and the predicted y value can be calculated as (y_i-y_{ipred}), and the residual sum of squares (RSS) can be calculated as:

$$RSS = (y_i - y_{ipred})^2$$

The total variation in the data is reflected in the corrected sum of squares (CSS), calculated as:

$$CSS = (y_i-y_{mean})^2$$

where: y_{mea} is the mean value of y.

The model sum of squares (MSS) is the portion of the total variation accounted for by the regression, i.e.

$$MSS=CSS-RSS$$

The coefficient of determination (r^2) is the proportion of the variation accounted for by regression, and is given by the ratio of the model sum of squares to the corrected sum of squares, i.e.

$$r^2 = MSS/CSS$$

In the case of linear regression, the coefficient of determination is equivalent to the square of the correlation coefficient.

The Standard Error was defined as follows:

The Standard Error of estimate (SE) gives a measure of the deviation of the calibration curve (and hence predicted y values) for the calibration data (and hence the actual y values). It is given by:

$$SE = (RSS/(n-p-1))$$

where: n is the number of data points in the calibration (the origin is counted as a data point if the curve is forced through zero).

p is the degree of polynomial being fitted by regression (i.e. 1 for linear to 6 for sexic).

The coefficient of determination and standard error for each analyte for each of the calibration types is listed in Table 22.

Table 22 Descriptive Statistics for Regression Calibration Curves

	Linear		Quadratic		Cubic	
Compound	Coefficient of Determination	Standard Error	Coefficient of Determination	Standard Error	Coefficient of Determination	Standard Error
HCBD	0.9843	6.12	0.9998	0.8619	0.9999	0.4945
HCB	0.9937	4.507	0.9998	0.9516	0.9999	0.8285
PCB 28	0.9994	0.4032	0.9994	0.4644	0.9994	0.5641
PCB 53(Surr)	0.9979	0.4887	0.9994	0.3016	0.9999	0.1488
PCB 52	0.9967	0.5692	0.9988	0.4001	0.9997	0.2397
Aldrin	0.9932	1.429	0.9999	0.189	1.0000	0.1631
op DDE	0.9974	0.8829	0.9999	0.2381	0.9999	0.2776
PCB 101	0.9964	0.6997	0.9991	0.4058	0.9997	0.2699
pp DDE	0.9938	2.052	0.9996	0.6304	1.0000	0.209
PCB 118	0.9994	0.3559	0.9994	0.4101	0.9999	0.2044
PCB 153	0.9983	0.5516	0.9992	0.4515	0.9999	0.1695
PCB 105	0.9940	1.436	0.9997	0.3597	0.9999	0.2437
PCB 138	0.9991	0.5052	0.9993	0.5174	1.0000	0.1058
PCB 156	0.9961	1.526	0.9994	0.7173	0.9997	0.5516
PCB 180	0.9995	1.944	0.9997	0.4465	0.9999	0.3502
α HCH	0.9858	1.775	0.9999	0.1523	0.9999	0.1779
β HCH	0.9971	1.151	0.9998	0.3507	0.9999	0.3194
γ HCH	0.9934	2.07	0.9997	0.5421	1.0000	0.1888
ε HCH	0.9993	1.242	0.9995	1.126	0.9996	1.3
Endo A	0.9950	1.984	0.9994	0.7888	1.0000	0.1831
Dieldrin	0.9924	2.181	0.9996	0.6073	1.0000	0.2173
Endrin	0.9902	2.172	0.9998	0.3403	0.9998	0.401
Endo B	0.9929	2.067	1.0000	0.1252	1.0000	0.09126
PP DDD	0.9807	2.812	0.9997	0.4131	1.0000	0.09551
OP DDT	0.9961	1.106	1.0000	0.132	1.0000	0.08019
PP DDT	0.9905	1.731	0.9999	0.2286	0.9999	0.2284
Bifenthrin	0.9995	1.944	1.0000	0.4394	-	-
Cis Permethrin	0.9998	0.6121	0.9998	0.7066	-	-
Trans Permethrin	0.9995	1.106	0.9998	0.7329	-	-

The mean % deviation of the 10 and 90 % calibration check standard from the nominal concentration for each analyte with a pre and post analysis calibration by the different methods of curve fit are listed in Table 23.

Table 23 Mean % Deviation of Calibration Check Standards Using Different Calibration Curves

	Linear		Quadratic		Cubic		Point to Point Linear		Cubic Spline	
Compound	10 %	90%	10 %	90 %	10 %	90 %	10 %	90 %	10 %	90 %
HCBD	6.85	-4.45	0.50	-5.47	0.73	-5.47	1.02	-4.15	-6.12	-8.04
HCB	5.83	-4.96	1.25	-6.84	0.93	-5.31	1.34	-4.3	-2.83	-7.71
PCB 28	1.09	-3.06	0.13	-3.01	0.24	-3.41	1.58	-3.44	-6.50	-6.51
PCB 53(Surr)	3.87	-5.36	0.89	-5.71	0.73	-4.45	1.42	-4.45	-4.96	-4.24
PCB 52	4.28	-5.22	1.34	-6.74	0.97	-2.52	0.96	-4.98	-3.42	-5.73
Aldrin	0.58	-0.38	0.21	0.44	0.52	-0.32	0.47	-1.14	-1.57	-0.51
op DDE	0.57	-1.39	0.01	-0.76	0.18	-1.82	1.06	-1.82	-0.97	-2.15
PCB 101	4.67	-1.04	0.70	-2.34	0.63	-0.70	0.60	-0.70	0.57	-2.09
pp DDE	4.06	1.09	0.28	1.79	0.35	-0.49	0.42	0.09	0.16	-0.26
PCB 118	5.29	-0.94	0.73	-0.98	1.09	1.77	0.12	-0.47	1.47	0.16
PCB 153	3.57	-3.79	0.91	-4.55	0.67	-0.88	0.75	-3.23	-3.42	-2.31
PCB 105	2.09	-3.78	0.27	-1.83	0.40	-3.50	1.36	-3.56	-2.28	-1.99
PCB 138	2.58	-3.82	0.91	-3.55	0.87	-0.32	1.72	-3.11	-1.37	-1.33
PCB 156	1.83	-0.41	0.70	0.69	1.03	2.95	1.21	0.23	-1.73	0.82
PCB 180	0.53	-6.91	0.85	-6.15	1.19	-4.4	0.66	-6.23	-3.27	-5.93
α HCH	-1.36	-4.27	0.93	-1.17	0.81	-1.55	0.82	-3.32	-4.23	-1.81
β HCH	0.25	-8.17	2.47	-7.18	1.03	-4.31	0.42	-7.81	-2.87	-7.10
γ HCH	1.86	-3.39	0.34	-1.29	0.46	-3.39	0.27	-3.32	-1.47	-3.19
ε HCH	2.30	-3.42	1.58	-3.51	1.87	-3.14	1.06	-2.96	-2.71	-2.86
Endo A	5.49	-1.93	0.70	-1.84	0.67	-2.08	1.34	-1.98	-1.57	-2.02
Dieldrin	3.27	-2.02	0.57	-1.35	0.47	-2.09	1.34	-2.06	-5.4	-12.0
Endrin	3.13	-1.38	0.19	-2.58	0.30	-1.39	0.96	-1.49	-7.3	-14.6
Endo B	2.78	-2.58	0.53	-1.79	0.67	-2.54	0.75	-2.69	-1.89	-2.54
PP DDD	4.96	-1.79	0.74	-1.91	0.82	-1.95	1.03	-2.01	-1.47	-1.86
OP DDT	2.36	-1.91	0.28	-1.87	0.21	-1.86	0.58	-2.01	-1.09	-1.86
PP DDT	2.17	-2.55	0.61	-1.51	0.34	-2.62	0.42	-2.72	-1.84	-2.66
Bifenthrin	1.36	-4.09	2.07	3.04	ND	ND	0.71	-3.65	-3.15	-4.42
Cis Permethrin	127	-6.57	0.98	-1.36	ND	ND	1.30	-6.43	-2.45	-6.70
Trans Permethrin	1.83	-5.81	1.03	-1.27	ND	ND	1.23	-5.30	-2.73	-5.71

The descriptive statistics indicated that a linear curve fit is not appropriate for the majority of analytes tested, poor coefficient of determinations (<0.9995) and high standard errors (>1.0) are observed for most of the analytes. Analysis of the 10 and 90 % calibration standards also indicated that linear curve fits are not appropriate, with significant positive bias at low concentrations and negative bias at high concentrations being observed. The clearest indication that a linear curve fit is not appropriate is provided by visual inspection of the calibration graphs. A quadratic curve fit of HCBD is shown in Appendix 8.

The descriptive statistics indicated that a quadratic curve fit describes the detector response of the majority of analytes extremely well with coefficient's of determination >0.9995 and standard errors <1.0. Check standards also indicated that a quadratic curve fit is appropriate for the majority of analytes. Visual inspection of the calibration graphs also supported the use of a quadratic curve to describe the detector response for the analytes.

The XCHROM data analysis software was unable to fit a cubic curve to the detector responses for bifenthrin, cis Permethrin, and trans permethrin,. For all the other analytes the descriptive statistics indicated that a cubic curve fit described the detector response better than any of the other curve fits investigated. The calibration bias of the 10 and 90 % check standards is similar for both the quadratic and cubic curve fits. Visual inspection of the calibration graph indicated that a cubic fit is appropriate for the data.

Descriptive statistics are not appropriate for either the point to point linear or the cubic spline calibration fits. Visual inspection of these curves indicated that both of these curve fits accurately reflected the detector response. Analysis of the 10 and 90 % check standards indicated that both of these techniques describe the detector response for all of the analytes well. The bias is greater for these techniques than for quadratic and cubic curve fit. To obtain high quality data from an interpolative curve fit it is essential that every point on the curve be known with high degree of certainty. If one point is "out" slightly due to an error in preparation it will have a dramatic effect on all concentrations determined between it and adjacent points. In contrast extrapolative curve fits tend to minimise the errors associated with an individual calibration point. This reduction in dependence on the individual analysis gives the extrapolative curve fits a significant advantage for a routine method. The curve fit types listed in Table 24 for each individual analyte was used in all subsequent determinations.

In order to minimise errors from extrapolation of these calibration curves the lowest and highest calibration standards should be used as the limits of quantification.

Table 24 Optimum Curve Fits

Compound	Curve Type	COMPOUND	Curve Type
HCBD	Cubic	α HCH	Quadratic
HCB	Cubic	β HCH	Cubic
PCB 28	Quadratic	γ HCH	Quadratic
PCB 53(Surr)	Cubic	ε HCH (SURR)	Cubic
PCB 52	Cubic	THE	Linear
Aldrin	Cubic	Endo_A	Quadratic
THE (INT STD)	Linear	Trans Nonachlor	Quadratic
op DDE	Cubic	PCB 112 (IntStd)	Linear
PCB 101	Cubic	Dieldrin	Quadratic
PCB 112 (INT STD)	Linear	Endrin	Cubic
pp DDE	Cubic	Endo_B	Quadratic
PCB 118	Quadratic	pp DDD	Quadratic
PCB 153	Cubic	op DDT	Cubic
PCB 105	Quadratic	Bifenthrin	Quadratic
PCB 138	Cubic	PCB 198 (INT STD)	Linear
PCB 156	Quadratic	Cis Permethrin	Quadratic
PCB 180	Cubic	Trans Permethrin	Quadratic
PCB 198 (INTSTD)	Linear	DCBP (INTSTD)	Linear
DCBP(INT STD)	Linear		

3.2.5 Gas Chromatography Mass Spectrometry

Gas chromatography mass spectrometry is a highly sensitive and specific technique which is widely used in environmental analysis. Historically GC/MS instruments were large, costly, highly specialised instruments restricted in use to confirmation identification of analytes. Recent improvements in instrument hardware have a led to the introduction of a variety of "benchtop" mass spectrometers. These GC/MS systems are relatively inexpensive, small and simple to operate.

Available for use in this study was a VG Trio 1 Mass spectrometer coupled with a HP 5890 Gas Chromatograph and HP 7673 Autosampler. The performance of this instrument was evaluated in a variety of ionisation modes to determine whether it offered superior performance to an electron capture detector for the routine determination of target analytes.

3.2.5.1 Electron Impact Ionisation

Following separation of complex mixtures by gas chromatography analytes are passed via a GC column through a heated interface called the ion block into the mass spectrometer. Within this ion block, held at earth potential, ionisation occurs.

Ionisation is achieved by an electron beam which travels between a tungsten filament and a trap. The filament is mounted outside the ion block eliminating contact with a hot surface on which the sample might decompose. The trap is behind a shield resulting in minimal field inside the ion block. The electrons enter the ion block from the filament and pass through the shield to the trap through small holes. A magnetic field produced by permanent magnets runs parallel to the electron beam to focus the electrons. These focused electrons bombard the analytes emerging from the GC column. This bombardment results in ionisation of analyte molecules by removal of one electron. This process of removing one electron leads to the formation of a positively charged radical ion containing one unpaired electron.

The electron beam energy required to remove an electron from an organic molecule is of the order of 10 eV. The energy of the bombarding electrons is typically set at 70 eV. The additional energy is dissipated in breaking bonds in the molecular ion causing the production of fragment ions.

These fragment ions are accelerated out of the ion block through an exit hole in the block.
The ions leaving the source are focused by a series of lenses (metal plates with circular holes set at variable DC voltages). The energy of these ions is reduced to a few electron volts passing through a plate at earth potential before entering the quadrupole mass analyser.

The quadrupole analyser consists of two sets of four accurately machined metal rods. A combination of RF and DC voltages on these rods restrict ions within a narrow range of masses (the resolution) to have a stable trajectory through this part of the analyser. Ions outwith the resolution range are rejected. The RF and DC voltages can be held constant to allow only one mass through in a time period .This is commonly known as selected ion recording (SIR). Alternatively the RF and DC voltages may be scanned through specified time periods to allow ions of a wide range of masses to pass through. The quadrupole filter can be set to scan between masses 1 to 1000 within one second.

From the quadrupole the ions of interest pass through a second earthed plate before being attracted by the conversion dynode, an Aluminium plate at -5000 Volts. The second earthed plate shields the ion from the dynode voltage until they have left the quadrupole. The ions are accelerated rapidly towards the dynode and on collision with the Aluminium produce electrons. These electrons are repelled by the dynode and attracted to a glass disk coated with an aluminised layer of phosphorus and held at 10000 Volts. The impact of these very energetic electrons on the phosphorus produces photons of light which are detected by a photomultiplier. Inside the photomultiplier the photons are converted back into electrons and this electron current is amplified to produce the detector output. The level of amplification is controlled by the photomultiplier voltage.

The mass spectrometer and gas chromatograph are fully computer controlled with all aspects of GC oven programming and mass spectrometer tuning handled via the computer software. Data collection and analysis are also controlled via the software, this allows construction and analysis of total ion chromatograms, single ion chromatograms, and multiple ion chromatograms. User generated libraries of target compound retention times and mass spectra can be generated and the software programmed to automatically search previously collected data for target compounds. Peak integration and analyte quantification can be programmed automatically via the software.
The software contains the NBS library of mass spectra of approx. 55000 organic compounds, this library can be automatically searched and unknown spectra compared to library spectra. This offers the potential of qualitative identification of unknown compounds in samples.

3.2.5.2 Full Scan Electron Impact Ionisation

Preliminary tests to assess the sensitivity, precision, accuracy and ease of use of GC/MS in the scanning Electron Impact (EI) mode as a method of detecting the target analytes.

All standards were prepared using the techniques described previously in section 3.2.1. The GC analysis was performed using the optimised conditions determined in section 3.2.3.3. For safety reasons VG do not recommend the use of hydrogen as a carrier gas with their mass spectrometers and therefore helium was used as the carrier gas in all tests. Mass spectrometer operating conditions were as outlined in Table 25.

Table 25 Mass Spectrometer Operating Conditions

Gas Chromatograph : HP 5890	Instrument : VG Trio 1
Autosampler : HP 7673	Interface Temperature : 250 °C
Injection technique : On-Column	Source Temperature : 200 °C
Injection Volume : 1 ml	Ionisation Mode : Electron Impact
Injector temperature : 70 °C	Electron Energy : 70 eV
Carrier Gas : Helium, Constant Pressure	Electron Current : 200mA
Retention Gap : 1 M length, 0.53mm ID	Scan range : 50-550 AMU
Analytical Column :	Scan Time : 0.95 sec
Oven Temperature Program :	Interchannel Delay : 0.05 sec

A standard containing the target analytes at concentrations between 1 and 5ppm were analysed using the above conditions. Electron Impact mass spectra for each of the target analytes was obtained and the spectra compared with the NBS library spectra. Visual comparison of standard and library spectra indicate an excellent agreement between the mass spectra.

A series of standards containing DCBP and THE at a concentration of 500 ug/l as internal standards and the target analytes at the approximate concentrations of 10000, 700, 500 300, 200, 100 ug/l were prepared in iso-octane. These were analysed using the above GC/MS conditions. Inspection of the total ion chromatograms indicate that the peaks of on column levels of 500pg of individual analytes are obscured by background noise and or column bleed. At lower concentrations it became necessary to look at ion chromatograms of individual masses characteristic of each compound. The minimum amount which could be detected using this technique varied from compound to compound.

It was estimated that compounds similar to HCB which give strong molecular ion fragments with minimal fragmentation could be detected at 50pg on column. Most compounds tested did not produce strong molecular ion fragments and 100 -150 pg on column was estimated as the minimum detectable amount. It should be noted that this was the minimum detectable amount using the most sensitive ion chromatogram. To obtain library search full scan spectra concentrations approximately 5 fold (i.e. 500pg) were required.

Characteristic ions for each analyte are listed in Table 26. Internal standardisation calibration graphs were prepared using the most intense ion for each analyte. Using the descriptive statistics discussed in section 3.2.4.3 a quadratic curve fit was found to most accurately describe the detector response over this range.

Table 26 Fragment Ions Suitable for Quantitation with Electron Impact Ionisation Mass Spectrometry.

Compound	Characteristic Ions m/z	COMPOUND	Characteristic Ions m/z
HCBD	222.75, 224.75, 226.73	α HCH	216.77, 218.75, 220.73
HCB	281.70, 283.70, 285.69	β HCH	216.77, 218.77, 220.75
PCB 28	255.83, 257.83, 259.81	γ HCH	216.77, 218.75, 220.75
PCB 52	289.77,291.75, 293.75	Endo_A	336.64, 338.64, 340.64
Aldrin	260.69, 262.75,264.75	Dieldrin	260.69, 262.63, 264.63
op DDE	245.91, 247.91, 317.81	Endrin	342.67,344.67, 346.67
PCB 101	323.70, 325.69, 327.69	Endo_B	336.66, 338.66, 340.66
pp DDE	245.89, 247.89, 317.81	pp DDD	234.84, 236.84,164.95
PCB 118	323.72,325.70,327.70	op DDT	234.84, 236.84,164.95
PCB 153	357.64, 359.64, 361.64	pp DDT	234.84, 236.84, 164.95
PCB 105	323.70,325.70,327.69	Bifenthrin	180.95, 182.02,164.95
PCB 138	357.66, 359.64, 361.64	Cis Permethrin	182.92, 183.97, 164.91
PCB 156	357.64, 359.64, 361.64	Trans Permethrin	182.94, 184.0, 164.91
PCB 180	391.59,393.58,395.58		

The 100 and 700 ug/l standards were sequentially analysed 12 times over a forty eight hour period. The peak heights of the characteristic ions of each analyte were normalised by dividing by the peak height of the characteristic ion of the analyte with the peak height of the characteristic ion of DCBP for Fraction 1 compounds and THE for Fraction 2 compounds. The percent RSD of the internal standard normalised peak heights are listed in Table 27.

Table 27 Reproducibility of Internal Standard Normalised Peak Heights

Compound	100 ug/l Std % RSD	700ug/l Std % RSD	Compound	100ug/l Std %RSD	700 ug/l Std % RSD
HCBD	13.4	8.2	α HCH	11.2	5.1
HCB	8.2	4.6	β HCH	16.8	6.2
PCB 28	19.3	8.6	γ HCH	10.7	4.2
PCB 52	16.9	8.9	Endo A	12.6	6.7
Aldrin	18.0	10.3	Dieldrin	12.6	8.4
op DDE	20.4	10.3	Endrin	20.6	16.4
PCB 101	20.3	8.5	Endo B	18.2	15.4
PCB 112	18.3	8.0	pp DDD	16.7	11.6
pp DDE	17.6	9.3	op DDT	15.9	13.4
PCB 118	20.3	7.8	Bifenthrin	21.6	12.6
PCB 153	21.8	8.1	Cis Permethrin	14.9	4.3
PCB 105	22.6	9.0	Trans Permethrin	13.3	4.7
PCB 138	21.4	8.1			
PCB 156	24.9	7.6			
PCB 180	20.5	12.4			

The precision achieved with the mass spectrometer in scanning mode is much poorer than the precision achieved from the Electron Capture Detector.

The only advantage of full scan EI GC/MS over GC/ECD as a detection technique is that it gives unequivocal identification of the target analytes and offers the possibility of identifying unknown contaminants which may be present in the sample extract. The major disadvantage of EI GC/MS is its lack of sensitivity and poor reproducibility. Full scan EI GC/MS is 50 fold less sensitive than GC/ECD and 5 times less reproducible. The poor sensitivity makes this technique unsuitable for routine monitoring and limits its use to special investigations or the confirmation of exceptionally high results.

3.2.5.3 Electron Impact Ionisation Selected Ion Recording

As indicated in section 3.2.5.1 the quadrupole can be programmed to allow only a single mass or a very small mass range through to the detector in a given time period. This technique is known as selected ion recording (SIR). By increasing the amount of time favourable for a selected mass to pass through the quadrupole to the detector the sensitivity of the MS can be increased. The Trio 1 allows the selection of up to 99 masses or mass ranges to be acquired over separate time intervals. The use of SIR in the Trio 1 is controlled by 5 variables: the mass, the mass span, the dwell time, the interchannel delay time and the retention window.

The Mass Span defines the mass of the fragment of interest. For highest sensitivities the exact mass of a fragment to 2 decimal places should be entered. The exact mass can be determined by analysing a standard in full scan mode immediately prior to programming for SIR.

The span is a small mass range centred around the "selected mass" to be scanned . The span value applied to each channel depends on how accurately the selected mass assignment reflects the value actually detected by the mass spectrometer , the smaller the span the higher the sensitivity. When a small span is used there is a risk of tail off in sensitivity due to the mass scale drifting.

The interchannel delay is the time allowed for the mass spectrometer to reset when switching between masses. It is normal to set this to low value, normally 0.01-0.1 secs. For large differences in mass between the selected masses a larger value will be required. The dwell time is the optimum time for a particular mass to reach the detector. Long dwell times give a higher signal to noise ratio. High dwell times should be avoided as too few points are collected across the chromatographic peak resulting in significant errors in quantification. It should be Noted that the selected masses are sequentially cycled e.g. monitoring masses x,y and z each with a dwell time of 0.5 sec and interchannel delay of 0.02 sec is undertaken by monitoring mass x from 0 - 0.5secs, mass y from 0.52 -1.02 secs, mass z from 1.04 to 1.54 secs, mass x from 1.56 to 2.06 secs . when a large number of masses are selected or if the dwell time is very long it is possible to completely miss peaks. To accurately quantify a peak it is necessary to collect from 10 to 20 points describing the peak. The retention window is the time period set to select for specified masses. Use of retention time windows allows small numbers of masses to be selected at different time period throughout the chromatographic analysis. The use of retention time windows is essential if only a small number masses are to be selected at any one time.

Using the exact masses and retention times determined in section 3.2.5.2 SIR programmes for Fraction 1 and Fraction 2 standards were prepared as given in Table 28. Using these programmes a series of standards containing the target analytes in iso-octane at the concentrations 500, 300, 200, 100, 50, 20, and 5 ug/l, and containing DCBP and THE as internal standards at 500 ug/l were analysed. Calibration graphs were prepared for each analyte. In addition the 500 and 20 ug/l standards were analysed 12 times over a 48 hour period. The peaks from these standards were internal standard normalised and the %RSD for each compound calculated

Table 28 Selected Ion Recording Program

Fraction 1							Fraction 2						
Channel No.	Compound	Mass (amu)	Span (amu)	Dwell (secs)	Inter chan Delay	Time (mins)	Chann I No.	Compound	Mass (amu)	Span (amu)	Dwell (secs)	Inter chan Delay	Time (mins)
01		222.75				7.5	01		216.77				16.5
02	HCBD	224.75	0.1	0.09	0.01	↓	02	α HCH	218.75	0.1	0.09	0.01	↓
03		226.73				9.5	03		220.73				18
04		281.70				16.5	04		216.77				18
05	HCB	283.70	0.1	0.09	0.01	↓	05	β HCH	218.77	0.1	0.09	0.01	↓
06		285.69				19.5	06		220.75				22
07		255.83				23.0	07		216.77				18
08	PCB 28	257.83	0.1	0.09	0.01	↓	08	γ HCH	218.75	0.1	0.09	0.01	↓
09		259.81				25	09		220.75				22
10		289.77				26	10		336.64				35
11	PCB 52	291.75	0.1	0.09	0.01	↓	11	Endo_A	338.64	0.1	0.09	0.01	↓
12		293.75				28	12		340.64				37
13		260.69				27.5	13		377.63				37.5
14	Aldrin	262.75	0.1	0.09	0.01	↓	14	Dieldrin	379.63	0.1	0.09	0.01	↓
15		264.75				29.5	15		381.63				40.5
16		315.83				34	16		342.67				41
17	OP DDE	317.81	0.1	0.09	0.01	↓	17	Endrin	344.67	0.1	0.09	0.01	↓
18		319.81				36	18		346.67				42.5
19		323.70				34	19		336.66				42.5
20	PCB 101	325.69	0.1	0.09	0.01	↓	20	Endo_B	338.66	0.1	0.09	0.01	↓
21		327.69				37	21		340.66				44
22		315.81				38	22		234.84				43.5
23	PP DDE	317.81	0.1	0.09	0.01	↓	23	pp DDD	236.84	0.1	0.09	0.01	↓
24		319.81				40	24		164.95				45
25		323.72				42	25		234.84				43
26	PCB 118	325.70	0.1	0.09	0.01	↓	26	op DDT	236.84	0.1	0.09	0.01	↓
27		327.70				45	27		164.95				45
28		357.64				44	28		234.84				47
29	PCB 153	359.64	0.1	0.09	0.01	↓	29	pp DDT	236.84	0.1	0.09	0.01	↓
30		361.64				46.5	30		164.95				50
31		323.70				45	31		164.95				55
32	PCB 105	325.70	0.1	0.09	0.01	↓	32	Bifenthrin	180.95	0.1	0.09	0.01	↓
33		327.69				47	33		182.02				58
34		357.66				47	34	Cis	182.92				69
35	PCB 138	359.66	0.1	0.09	0.01	↓	35	Permethrin	183.97	0.1	0.09	0.01	↓
36		361.66				50	36		164.91				73
37		357.64				55	37	Trans	182.91				69
38	PCB 156	359.64	0.1	0.09	0.01	↓	38	Permethrin	184.0	0.1	0.09	0.01	↓
39		361.64				57	39		164.91				73
40		391.59				57							
41	PCB 180	393.58	0.1	0.09	0.01	↓							
42		395.58				60							

Using this technique improved sensitivity was observed for all of the compounds analysed. Minimum detectable quantities were estimated as between 6-20 pg on-column for each analyte. Calibration graphs between 20-500pg on column were prepared for each analyte using the descriptive statistics described earlier. Quadratic curve fits were found to best describe the detector response.

Table 29 % RSD of standards analysed by SIR

Compound	20 ug/l Std % RSD	500ug/l Std % RSD	COMPOUND	20ug/l Std %RSD	500 ug/l Std % RSD
HCBD	4.3	3.6	α HCH	2.1	3.2
HCB	6.4	3.4	β HCH	1.9	3.3
PCB 28	5.9	4.0	γ HCH	2.6	4.2
PCB 52	6.1	5.1	Endo A	12.6	3.9
Aldrin	6.4	3.7	Dieldrin	8.6	3.8
op DDE	6.8	4.9	Endrin	8.4	6.8
PCB 101	8.6	3.9	Endo B	8.6	5.1
pp DDE	9.1	6.5	pp DDD	9.3	6.8
PCB 118	5.2	4.2	op DDT	8.5	7.2
PCB 153	5.7	3.9	pp DDT	10.1	7.4
PCB 105	5.9	4.3	Bifenthrin	6.0	3.4
PCB 138	6.1	4.6	Cis Permethrin	5.2	4.2
PCB 156	6.7	4.3	Trans Permethrin	5.4	4.0
PCB 180	5.9	3.9			

The results in selected ion recording mode are a significant improvement on the precision achieved in scanning mode, the results approach the precision achieved with the Electron Capture Detector.

EI SIR offers 2 major advantages over EI scanning mode; lower detection limits, and improved precision. These advantages are offset by 2 disadvantages: less spectral information, with no facility for identification of unknown contaminants and more complex programming.
The EI SIR programme requires that analytes should have reproducible retention times, otherwise they may not elute in their specified detection window. The time required to program EI SIR and the possibility of failure to detect analytes due to retention time drift make the technique unsuitable for routine scanning for a wide variety of compounds, The lower detection limits achieved make it the technique of choice for confirmation analysis.

3.2.5.4 Negative Chemical Ionisation

Although Electron Impact ionisation is the most commonly used ionisation method in mass spectrometry there are a variety of alternative methods. Chemical Ionisation (CI) is a frequently used alternative method of ionisation. CI is regarded as a "soft ionisation" technique, because the molecular or quasi-molecular, ions are formed with a much smaller excess of internal energy than in EI ionisation. This lower excess energy leads to less fragmentation and greater abundance's of molecular ions. Negative ion Chemical Ionisation (NCI) has seen widespread use for the structure elucidation of compounds having a high electron affinity, such as halides, aromatic nitro compounds and quinones.

In CI a reagent gas, commonly ammonia, methane or isobutane is 'leaked' into the ion source. These large numbers of reagent gas molecules produce much higher pressures, typically of about 1 torr, in the source. The large numbers of reagent gas molecules are ionised by electron impact. The ionised reagent gas molecules undergo a large number of collisions with each other and unreacted reagent gas until they posses the thermal energies corresponding to equilibrated ground-state species at the temperature of the ion source. During the equilibration two main types of ion analyte reactions may occur : 1) Electron capture, this involves the capture of an electron by a molecule which has a vacant, low-energy orbital. Compounds with double bonds, sulphur, phosphorus and halogens except fluorine commonly undergo these reactions. 2) Reactant - ion chemical ionisation, this occurs by reaction of a compound with thermally equilibrated, negatively charged reagent gas ions. A variety of ion/molecule reactions may occur but the commonest is proton abstraction.

NCI offers considerable advantages for environmental analysis. In particular a limited fragmentation and high degree of specificity. This produces simpler chromatograms that screen out compounds of lesser interest and allow heavier masses to be used for quantification. This improves sensitivity.

To assess the sensitivity, precision, accuracy and ease of use of GC/MS in scanning NCI mode using methane as a reagent gas as a method of detecting the target analytes.

A standard containing the analytes at approximately 1 ppm was prepared using the techniques described previously in section 3.2.4. This standard was analysed using the NCI GC/MS conditions given below in Table 30.

Table 30 Negative Chemical Ionisation Mass Spectrometer Operating Conditions

Gas Chromatograph : HP 5890 Autosampler : HP 7673 Injection technique : On-Column Injection Volume : 1 ml Injector temperature : 70 °C Carrier Gas : Helium, Constant Pressure Retention Gap : 1 M length, 0.53mm ID Analytical Column : Oven Temperature Program : 70 °C for 1 min, 70 - 180 at 20 °C/min, Hold at 180 °C for 2min, 180 °C - 230 °C at 1.2 °C/min, 230-280 °C at 3.5 °C/min Hold at 280 °C for 15 min	Instrument : VG Trio 1 Interface Temperature : 250 °C Source Temperature : 120-200 °C Ionisation Mode : Electron Energy : 70 eV Electron Current : 200mA Scan range : 50-550 amu Scan Time : 0.95 secs Interchannel Delay : 0.05 sec Reagent Gas: Methane

To determine the optimum conditions the source temperature was increasingly raised in the range shown above. After optimisation of the NCI conditions a series of standards containing DCBP and THE as internal standards at 500 ug/l and the target analytes at the following approximate concentrations 500 300, 200, 100, 50, 20, 5 and 1 ug/l were analysed.

Negative CI demonstrates good sensitivity for most of the target analytes despite a number of problems obtaining these results. The technique produces a large amount of ion burn on the repellor and focus plates as a result of which the source requires frequent cleaning. Because of this the CI technique is often referred to as a 'dirty' technique. The source is operated at higher pressures than in EI and as a result the filament burns out frequently. Difficulty was experienced in tuning the mass spectrometer and as a result the tuning variations gave differences in ionisation efficiency, sensitivity and the compound mass spectra.

The source temperature had a marked effect on the sensitivity and the mass spectra obtained. Lower source temperatures produced much less fragmentation and favoured the production of molecular ions giving better sensitivity. 120 °C was determined as the lowest temperature at which the Trio 1 source could be maintained. Lower set source temperatures drifted upwards as the filament was used and the GC oven temperature increased. When source temperatures below 120 °C were selected the drift could not be controlled and was not reproducible. The optimum temperature was selected as 120 °C.

Negative chemical ionisation mass spectra for each of the target analytes were obtained using these optimised conditions. The molecular ions and important fragmentation ions suitable for quantitation are listed in Table 31. Difficulty was experienced in obtaining molecular ion spectra for PCB 28, ions at m/z 35 and 37 were found to give much better sensitivity.

Table 31 Fragment Ions Suitable for Quantitation with Negative Chemical Ionisation Mass Spectrometry.

Compound	Characteristic Ions m/z	COMPOUND	Characteristic Ions m/z
HCBD	70,118	α HCH	71,73,257
HCB	284,286	β HCH	71,73,257
PCB 28	35,37	γ HCH	71,73,257
PCB 52	35,37	Endo_A	406,237
Aldrin	237,235	Dieldrin	237,235
op DDE	246,248	Endrin	237,346
PCB 101	326,324	Endo_B	406,237
pp DDE	281,318	pp DDD	71,73
PCB 118	326,324	op DDT	71,73
PCB 153	360,362	pp DDT	71,73
PCB 105	326,324	Bifenthrin	NA
PCB 138	360,362	Cis Permethrin	35,207
PCB 156	360,362	Trans Permethrin	35,207
PCB 180	394,360		

As indicated difficulties were experienced when carrying out the calibration and reproducibility tests. During the test runs the instrument sensitivity would change by an order of magnitude. A sensitivity of large peaks for a 1µg/l standard would deteriorate after the analysis of 6 samples to difficulty in detecting peaks from a 100µg/l standard. Discussions with the manufacturers indicated that the reagent gas (methane) might be the source of the problem. Methane is traditionally thought of as a "dirty" reagent gas. It was suggested by the manufacturer that the source was quickly becoming contaminated. This contamination reduced the transmission of ions to the quadrupole resulting in a dramatic loss in sensitivity. Ammonia is considered a much cleaner reagent gas, and the manufacturer suggested that we should use ammonia as the reagent gas in preference to methane.

Initial tests with Ammonia were encouraging, but a serious chemical accident involving the leakage of ammonia gas occurred during the first overnight test. The Ammonia reagent gas was switched off and the room ventilated. Investigations with the manufacturer revealed rubber O rings in the reagent gas control valve. This was a serious design fault rendering the instrument unsuitable for use with Ammonia gas as recommended by the manufacturer. Despite modifications made by the manufacturer CRPB took the decision that without expensive gas controls and monitors Ammonia was unsuitable for routine laboratory use. No further tests were carried out with Ammonia as the

reagent gas. Personal contact with users of similar GC/MS systems confirmed the severe loss in sensitivity when methane was used as a reagent gas. Information on larger more sophisticated instruments indicated that they did not experience this problem. It was suggested that this was due to the superior gas control on these instruments.

The pressure inside the Trio 1 source cannot be accurately monitored and, therefore, it is difficult to reproduce the conditions inside the source from day to day. The reagent gas is bled into the Trio 1 source by a simple needle valve. Fluctuations in temperature and reagent gas pressure affected the amount of reagent gas entering the source and hence the sensitivity. The Trio 1 uses a combined EI/ CI source. When CI is being used a source exit plate with a smaller hole is used to help obtain the higher source pressure required. Larger instruments use a dedicated CI source with a much smaller internal volume.

As a result of this study and the subsequent discussions with the manufacturer these limitations in early Trio 1 instruments for the production of CI spectra were recognised by the manufacturer and design alterations made. Subsequent models e.g. the Trio 1000 and MD 800, have specialised CI inserts which reduce the internal volume of the source. Third party manufacturers (ASH Instruments) now provide kits which can be fitted to the Trio to enable better control of the source pressure. These developments were unavailable to this study.

Initial tests indicated that NCI potentially offered good sensitivity and specificity for the determination of the target analytes. The technique required a great deal of operator skill to optimise and obtain reliable results. The design of the available CI source was not suitable for the use of Ammonia as a reagent gas and could not be fully tested or optimised. NCI in its present form is considered unsuitable for the routine determination and confirmation of the target analytes.

3.3 Concentration Techniques

An important requirement in a multi-residue method is to be able to concentrate the extract by removal of excess solvent. This is a key step in any multi-residue method and may be performed many times during the analysis. Losses or degradation of an analyte during evaporation is a possibility in this step and can be a major source of error in the analysis scheme. A wide variety of techniques are used for solvent removal and the more common are considered and evaluated for use with a multi-residue analytical procedure.

1. Kuderna-Danish Evaporator Fitted with Micro Snyder Column.

This apparatus is routinely used with a water bath but can be used with an electromantle. The sample is concentrated into a small teat at the bottom of the apparatus. The solvent vapour is forced up into the micro Snyder column, where the glass beads reduce the velocity of the solvent vapour bringing it into contact with the condensing solvent. This method has been used for a number of years for a wide variety of multi-residue methods. It consistently gives high recoveries for a wide range of determinands. The major disadvantages are the care and time required to set up and use the apparatus. When used with water baths strict control must be exercised to avoid ingress of water at the joints. Close supervision is required to prevent evaporation to dryness. Determinands of medium and high volatility may be lost when extracts are reduced to less than 0.5 mls volume. Considered an excellent method of solvent reduction for experimental and research methods but too intricate and time consuming for use in routine analysis.

2. Rotary Evaporation.

One of the oldest and most widely used methods of solvent evaporation. It is used for a wide range of solvent types and volumes. Care must be taken to control the evaporation conditions since evaporation to dryness or a very small volume may result in losses of medium and highly volatile determinands. This method is an excellent means of reducing large volumes of solvent, however it is very time consuming as only one sample can be reduced in volume at a time. Because of the time required for each sample the technique is considered unsuitable as a routine multi-residue method.

3. Evaporation in a Stream of Dry Air or Nitrogen.

In this method the solvent is gently heated by either a water bath or an electromantle while a stream of either dry air or nitrogen is blown across the top of the sample. This apparatus is easily scaled up to provide simultaneous evaporation of a large number of samples and has found wide acceptance in PCB residue analysis. To achieve reproducible high recoveries care must be taken to optimise the evaporation rate. Evaporation to dryness or a small volume will result in losses of medium and high volatility determinands. This technique is slow and therefore usually restricted to volumes of extract less than 50 mls. A high degree of operator intervention is required to maintain optimum evaporation rate and prevent evaporation to dryness. This method is suitable for multi-residue

applications. The high degree of analyst intervention required limits its use to laboratories where sample throughput and efficiency are not major considerations.

4. Turbo Vap Concentration.

This technique is an automated version of evaporation in a stream of dry air or nitrogen. The samples are heated in a water bath and the solvent evaporated by high volumes of dry air blown across the surface of the sample in a gas vortex shearing technique. This approach speeds solvent evaporation through an improved gas/solvent interface giving a more efficient vapour removal. The evaporator directs the gas flow into the sample tubes at a precise angle. The vortex created by the blowing gas travels down the tube to the solvent surface, where it increases the gas solvent interface with the sample. Simultaneously the vortex creates an escape route for the solvent-saturated gas up through the centre of the vortex. The gas flow gently agitates the sample, rinsing the container walls and minimising loss of analytes.

Conclusion

All of the techniques described have been successfully used for various multi-residue methods. This study required the development of a fast, robust, modular method readily adaptable to routine use for analysis of large numbers of a variety of sample types. The TurboVap method was selected for further study as the method most likely to meet these requirements.

3.3.1 Optimisation of Solvent Reduction with the Turbo Vap Evaporator

The manufacturers manual gives details of the approximate conditions for optimum analyte recovery and gives details of the parameters which have the greatest effect on analyte recovery. The following experiments were carried out to optimise analyte recovery from the reduction of Hexane based extracts.

General Experimental Design.

To simplify method development the following analytes were selected as representative of the range of chemical and physical properties of the target analytes; HCBD, α HCH, HCB, γ HCH, ε HCH, PCB 28, PCB 53, aldrin, dieldrin, PCB 118, pp DDD, pp DDT, PCB 180, cis permethrin, trans permethrin. A standard prepared in hexane containing these compounds at approximately 40 μg/l was used to spike 50 ml portions of hexane. The spiked hexane was then reduced to 0.5ml using the TurboVap under various test conditions. The evaporated solutions were analysed by GC/ECD under the optimised conditions determined in section 3.2.3.

The mean % recovery and RSD were calculated for each analyte.

3.3.1.1 Optimisation of Gas Pressure

Using a Turbo Vap evaporator at a bath temperature of 30°C six 50ml portions of spiked hexane were evaporated to 0.5mls at a range of gas pressures.

The percentage recovery and relative standard deviation of each of the analytes and the time taken are listed in Table 32.

Table 32 Effect of Air Pressure on Concentration Efficiency.

Air Pressure	5 psi		10 psi		15 psi		20 psi	
Compound	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	68	3.73	71	3.88	73	4.52	70	3.80
α HCH	74	3.31	74	4.00	77	4.12	76	3.58
HCB	69	4.20	72	4.13	73	4.21	73	3.85
γ HCH	76	3.54	77	4.27	79	4.06	75	4.32
ε HCH	73	4.49	75	4.16	72	3.48	69	4.07
PCB 28	84	4.85	83	3.51	79	4.14	82	3.81
PCB 53	86	4.37	93	3.89	91	4.08	86	3.30
Aldrin	78	3.21	81	4.32	83	3.68	78	4.02
Dieldrin	83	3.46	85	3.03	83	3.63	79	3.95
Endrin	84	4.94	86	3.94	84	3.57	86	4.12
PCB 118	91	3.52	98	3.27	96	4.11	91	3.81
PP DDD	84	3.48	83	4.51	86	3.76	77	3.49
PP DDT	86	4.93	86	4.51	88	3.74	83	3.94
PCB 180	94	3.55	93	3.50	91	3.68	94	3.86
Cis Permethrin	91	3.88	93	3.60	94	2.83	85	3.49
Trans Permethrin	92	3.13	91	3.36	93	2.96	86	3.39
Time	23 minutes		20 minutes		17 minutes		16 minutes	

These recoveries are lower than expected from the manufacturers literature. The recoveries of the more volatile analytes HCBD, α HCH, HCB, and γ HCH are not acceptable. A typical multi-residue method involves 3-4 solvent removal steps. These results indicate that using a Turbo-Vap evaporator for solvent removal in a typical multi-residue method would result in losses of 70-80% for some determinands.

Gas pressure increases the evaporation rate, higher pressure gives a faster solvent removal, but does not significantly affect the analyte recovery. Only a small difference in recovery is observed between 15 and 20 psi gas pressure. 15 psi was selected as the optimum pressure setting.

3.3.1.2 Investigation on the Effects of the Addition of a Keeper.

A keeper is a solvent with a high boiling point, and a high affinity for the target analytes. A small volume (0.5ml) of keeper is added to the extract before solvent removal to reduce volatilisation of the determinands and improve recovery. The analytes are preferentially concentrated into the keeper minimising losses of the analytes. Keepers are routinely used in rotary evaporation techniques. Some examples of commonly used keepers are iso-octane, toluene, nujol and decane. For this study only iso- octane and decane were investigated, toluene and nujol were not tested because a) they could not be readily purchased in sufficient purity and b) the high polarity of these solvents was anticipated as likely to interfere with subsequent adsorption chromatography.

Using a Turbo Vap evaporator with the optimum conditions of pressure determined in the previous test and a bath temperature of 30 °C, six replicate 50ml portions of hexane spiked as described previously were evaporated to 0.5mls using test keepers. The keepers tested were 0.5mls of iso octane and decane.

The percentage recovery and relative standard deviation of each of the analytes and the time taken are given in Table 33.

Table 33. Effect of a Keeper on Concentration Efficiency

Compound	Iso Octane		Decane	
	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	82	3.97	81	4.16
α HCH	88	3.84	85	4.26
HCB	84	3.59	86	3.62
γ HCH	91	3.70	85	4.01
ε HCH	85	3.91	84	3.61
PCB 28	89	3.40	93	3.88
PCB 53	95	3.98	94	4.09
Aldrin	96	3.55	97	3.62
Dieldrin	92	4.28	95	3.29
Endrin	96	3.67	97	3.20
PCB 118	101	4.39	99	3.29
PP DDD	97	3.85	98	3.33
PP DDT	98	3.65	99	3.72
PCB 180	103	3.89	100	3.90
Cis Permethrin	102	4.19	103	3.37
Trans Permethrin	100	3.38	97	3.64

Addition of a keeper improves the recovery of most of the target analytes but the recoveries of the volatile analytes are still unacceptably low. No significant difference is observed between iso Octane and decane as keepers. Iso octane has the advantage of being a cheap, readily available, pure solvent. Decane is more expensive (10 times) and not readily available. Iso octane was chosen as the keeper.

3.3.1.3 Optimisation of Bath Temperature.

Using a Turbo Vap evaporator and the optimised conditions of pressure and keeper found in previous experiments, six replicate 50 ml portions of hexane spiked as per previous tests were evaporated to 0.5mls at room temperature and other set bath temperatures.

The percentage recovery and relative standard deviation of each of the analytes are given in Table 34. The results from the previous test carried out at 30 °C have been included in this table for comparison.

Table 34 Effect of Bath Temperature on Concentration Efficiency

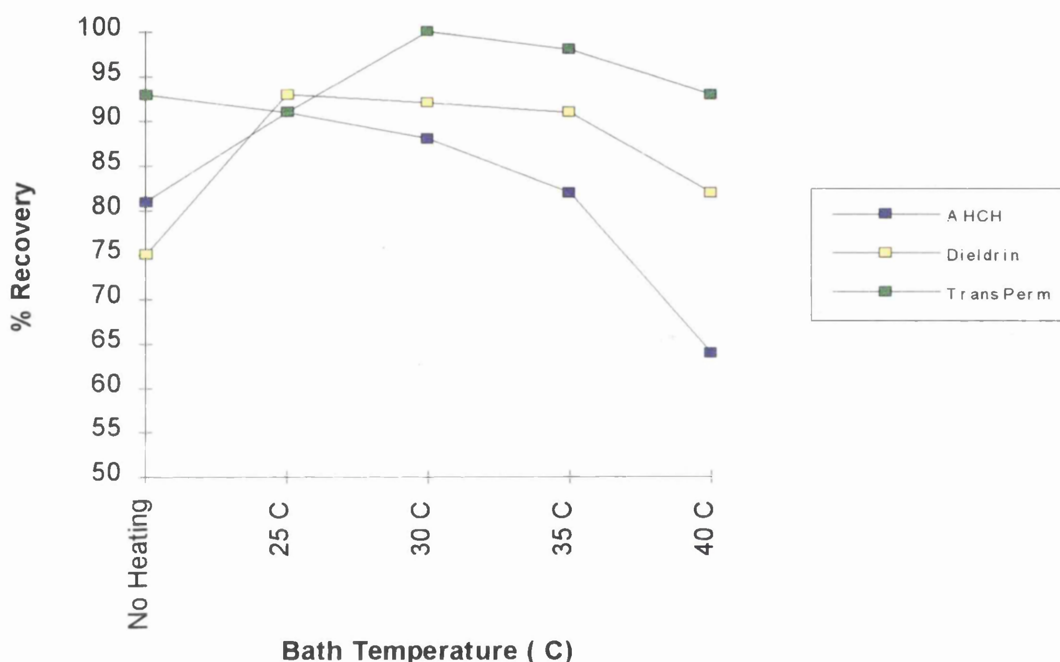
Bath Temperature	No Heating		25 °C		30 °C		35 °C		40 °C	
Compound	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	283	41.2	167	28.53	82	3.97	174	32.5	162	28.6
α HCH	81	4.35	91	4.78	88	3.84	82	4.38	64	4.53
HCB	76	4.22	86	4.31	84	3.59	77	4.05	56	3.94
γ HCH	85	4.46	85	3.71	91	3.70	87	3.52	66	3.00
ε HCH	77	4.60	93	4.18	85	3.91	76	4.15	59	4.19
PCB 28	87	4.0	96	3.35	89	3.40	87	4.06	71	4.03
PCB 53	84	4.18	93	4.19	95	3.98	93	4.29	83	5.01
Aldrin	76	4.20	95	4.42	96	3.55	97	4.30	74	4.52
Dieldrin	75	4.68	93	3.99	92	4.28	91	3.86	82	3.71
Endrin	77	4.43	98	4.04	96	3.67	95	3.76	87	4.87
PCB 118	79	4.40	97	4.44	101	4.39	99	3.74	86	3.67
PP DDD	85	4.0	98	3.69	97	3.85	96	4.39	89	4.66
PP DDT	88	3.63	99	3.74	98	3.65	98	3.97	88	4.18
PCB 180	89	3.70	98	4.34	103	3.89	98	3.56	90	3.25
Cis Permethrin	91	3.78	97	3.84	102	4.19	99	4.20	94	4.05
Trans Permethri	93	3.55	91	3.78	100	3.38	98	3.72	93	3.83

The TurboVap evaporator relies on a clean dry supply of air to evaporate the samples. While this test was being carried out the charcoal and silica gel traps which clean the air supply for the TurboVap evaporator became contaminated with hydrocarbons from the compressor. This led to "dirty" chromatograms. These interferences are most noticeable close to the HCBD peak where a large number of interfering peaks are detected. As a result elevated results are obtained for HCBD. The results for the other analytes are unaffected by this contamination.

Figure 6 shows a plot of percentage recovery of analytes of high (α HCH),medium (dieldrin) and low (trans permethrin) volatility against bath temperature. The results indicate a recovery maximum for all of these compounds between 25 and 30 °C. This maximum is least pronounced for the least volatile compounds.

The optimum water bath temperature was selected as between 25 and 30 °C.

Figure 6 Analyte Recovery Vs Bath Temperature



Although greatly improved these recoveries would result in significant losses of volatile analytes in a method which required multiple solvent reduction steps. A possible mechanism for the loss of these volatile lipophilic compounds is adsorption to the glass surface of the TurboVap tube as the extract is progressively evaporated. Earlier tests demonstrated that when extracts were allowed to evaporate to dryness severe losses of volatile analytes occurred (no data presented observation from previous test). Adsorbed analytes are likely to be lost directly from the glass surface. This type of loss would be greater for samples than for test solutions because of the higher concentrations of lipids. In an effort to overcome these losses a test was carried out stopping the evaporation process at regular intervals to rinse the walls of the TurboVap tube washing adsorbed analytes back into solution before they could be lost.

As in previous experiments six 50 ml portion of hexane were spiked with the target analytes, 0.5mls of Iso octane keeper and evaporated to 0.5mls. At approximately 10 mins intervals the TurboVap tube was removed from the evaporator and the inner walls rinsed using a Pasteur pipette with 1 -2 mls of hexane.

The percentage recovery of each of the analytes along with the relative standard are given in Table 34.

Table 34 Percentage Recovery of Target Analytes following Turbovap Evaporation With and Without hexane Rinsing.

Compound	With hexane Rinsing		Without hexane Rinsing	
	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	87	4.10	82	3.97
α HCH	92	3.95	88	3.84
HCB	88	4.29	84	3.59
γ HCH	90	3.97	91	3.70
ε HCH	86	3.68	85	3.91
PCB 28	91	4.04	89	3.40
PCB 53	97	3.96	95	3.98
Aldrin	95	4.38	96	3.55
Dieldrin	97	3.68	92	4.28
Endrin	98	4.85	96	3.67
PCB 118	99	4.24	101	4.39
PP DDD	100	3.58	97	3.85
PP DDT	99	4.04	98	3.65
PCB 180	100	3.92	103	3.89
Cis Permethrin	98	3.99	102	4.19
Trans Permethrin	103	4.17	100	3.38

No significant improvement in recovery is observed for the intermediate and low volatility analytes. A small improvement in recovery is observed for the high volatility analytes. Hexane washing results in only a slight improvement in recovery for a small number of analytes. The additional work involved in hexane washing is small and anticipating that losses were likely to be greater for real samples it was decided to include the surface washing as a routine precaution in the evaporation of samples.

3.3.1.4. Conclusions

TurboVap concentration is a fast and efficient method of reducing/concentrating hexane extracts. Target analytes with a wide range of physical and chemical properties can be quantitatively recovered when using this method of solvent reduction. This method is ideal for use in multi-residue methods where multiple concentration steps may be required.

The optimum conditions are outlined in Table 35.

Table 35: Optimum Evaporation Conditions.

Model	Zymark TurboVap Evaporator
Gas Pressure	15 psi
Keeper	Iso-Octane
Bath Temperature	25 -30 °C
Usage	Stop evaporation regularly and rinse tube walls with hexane .

3.4 Optimisation of Adsorption Chromatography Clean-Up Techniques

As discussed in section 2.3. the aim of an extract clean-up is to separate the target analytes from potentially interfering co-extractives which may be present in the sample extract. In section 2.3.1.6 it was identified that the following clean-up methods were suitable for the removal of different classes of interferences:

1. Normal phase chromatography using silica gel as the adsorbent for the separation of analyte classes.

2. Reverse phase adsorbents such as Aminopropyl bonded silica for the removal of gross contaminants such as lipids.

3. Normal phase chromatography using adsorbents such as alumina and florisil for the removal of gross contaminants such as lipids and co-elution contaminants such as phthalates and humic acids.

In the following investigations all of these clean-up up techniques were tested, optimised and evaluated in order to determine the appropriate clean-up strategy for each type of matrix.

3.4.1 Optimisation of the Separation of Analyte Classes using Silica Gel

The complex chromatograms of PCB/Organochlorine pesticide mixtures obtained in the analysis of environmental samples are characterised by a large number of peaks. As discussed in section 3.2.3 the complete separation of OCPs from the PCBs typically present in environmental samples by high resolution capillary columns is not at present possible. To minimise errors due to co-elution of compounds and mis-identification of peaks it is necessary to separate the majority of OCPs from the PCBs prior to the final determination. It is not necessary to separate HCBD and HCB from the PCBs as these compounds elute earlier than the other PCBs.

This separation of PCB's from OCPs has in the past been performed on adsorbents such as silica. The following series of tests were undertaken to optimise the separation of PCBs from OCPs.

3.4.1.1 Investigation of the Use of 3.5% Deactivated Silica Gel for the Separation of PCBs from Pesticides.

The procedure outlined in HMSO Method for the Examination of Water and Associated Materials (MEWAM) No. 13 for the separation PCBs and OCPs was used as an initial starting point.

Preparation of Reagents

1. Silica Gel

   Approximately 100g of silica was heated in a furnace at 500 °C for 4 hours. The silica was cooled in the furnace to approx. 200 °C then transferred to a dessicator where it was cooled to room temperature. 96.5 +/- 0.5 g of silica were transferred to a screw cap conical flask and 3.5 mls of deionised water added. The conical flask was sealed and shaken vigorously by hand for 2 mins before being transferred to a mechanical orbital shaker where it was shaken for a further hour prior to use. The silica was used on the day it was prepared.

2. Sodium Sulphate

   Approximately 50g of sodium sulphate was heated to 500 °C for 4 hours in a furnace. The sodium sulphate was cooled in the furnace to approx. 200 °C then transferred to a dessicator where it was cooled to room temperature. The cooled sodium sulphate was transferred to a screw a cap glass bottle for storage before use. Prepared sodium sulphate was not stored for more than 5 days prior to use.

3. Recovery Test Solution

   Using the techniques outlined previously a standard solution containing the following target analytes was prepared in hexane: HCBD, HCB, γ HCH, ε HCH, PCB 53,PCB 52, aldrin, PCB 101, PP DDE, dieldrin, endrin, PP DDT, PCB 180, cis permethrin, trans permethrin.

4. Internal Standard Solution

   Using the techniques outlined previously a standard solution containing trans heptachlor epoxide and decachlorobiphenyl was prepared in hexane.

Analytical Procedure.

1. A 10 mm diameter * 500mm length glass column fitted with a PTFE stopcock was plugged with a small piece of glass wool. The column and wool were rinsed with 10mls of acetone followed by 10mls of hexane.

2. 2g +/- 0.1g of deactivated silica was placed in the column with 0.2 +/- 0.1g of sodium sulphate added to the top. The column was tapped vigorously to ensure even packing.

3. Using a 1ml syringe 1ml of the recovery solution was placed on the column.

4. The 1ml was allowed to pass into the silica. When the meniscus of the recovery solution was observed passing into the sodium sulphate 15mls of hexane was added to the column. Care was taken to ensure that the column was never allowed to go dry.

5. The first 7ml portion of the eluate was collected in a 10ml test tube.

6. 25mls of 10% MTBE in hexane was added to the top of the column. Care was taken to avoid disruption of the silica bed.

7. The 7-40mls fraction was collected in a 50ml turbo vap tube.

8. Both fractions were reduced to 0.5mls using a tubovap concentrator under the optimum conditions determined in section 3.3.

9. Using 0.5mls of iso-octane both fractions were quantitatively transferred to GC vials for GC/ECD analysis. Internal standard solution was added, the vial sealed and shaken thoroughly.

10. Both extracts were analysed by GC/ECD using the optimum conditions outlined in section 3.2.

Replicates (6) were carried out . Mean recoveries for each of the analytes along with the RSD are presented in Table 36.

The results show that under the test conditions the PCBs are separated from most of the OCPs. HCBD, HCB, aldrin and the PCBs are quantitatively recovered in the fraction 1 extract and γ HCH, ε HCH, dieldrin, endrin, cis and trans permethrin are quantitatively recovered in the Fraction 2 extract. PP DDE and PP DDT are not successfully separated from the PCBs by this method. 85 % of PP DDE is recovered in the Fraction 1 extract with the PCBs and PP DDT evenly split between the two fractions. This split of PP DDE and PP DDT is not acceptable since identification and quantification of these compounds is difficult when they are present in the same extract. In the fraction 1 extract the identification and quantification of PP DDE and PP DDT is made further difficult by co-elution with PCBs. To quantify the PP DDE and PP DDT in Fraction 2 where they are clear of interference by PCBs the recovery needs to be improved to attain acceptable detection limits.

Table 36. Separation of PCBs from OCPs using 3.5% deactivated Silica Gel

	% Recovery			
Compound	0-7ml Fraction 1	RSD	7-40ml Fraction 2	RSD
HCBD	78	5.6	8	39.9
HCB	78	5.8	9	41.8
γ HCH	ND	-	93	4.6
ε HCH	1	-	101	4.7
PCB 53	86	7.8	9	33.8
PCB 52	88	9.5	12	43.7
Aldrin	88	5.8	11	25.0
PCB 101	84	5.7	9	37.1
PP DDE	85	12.6	23	38.9
Dieldrin	ND	-	99	4.6
Endrin	ND	-	100	2.7
PP DDT	50	11.9	50	12.0
PCB 180	98	6.5	ND	-
Cis Permethrin	ND	-	100	2.7
Trans Permethrin	ND	-	101	4.1

Where ND = Not Detected.

Further tests were carried to determine the exact volumes at which PP DDE and PP DDT eluted to attempt to obtain quantitative recoveries by cut volume manipulation.

3.4.1.2 Elution profile of PP DDE and PP DDT on a 3.5% Deactivated Silica Gel column.

Using the same test materials and method as in section 3.4.1.1 1ml portions of the recovery solution was taken through the separation procedure. The eluent were collected in 5 fractions instead of the two in the previous test. The fractions collected were i) 0-5ml ii) 5-7ml iii) 7-9ml iv) 9-20ml v) 20-40ml.

This procedure was repeated six times. The mean % Recovery and RSD in brackets of each analyte is listed in Table 37.

Table 37 % Fractionation of OCPs and PCBs on a 2g 3.5% deactivated Silica Column.

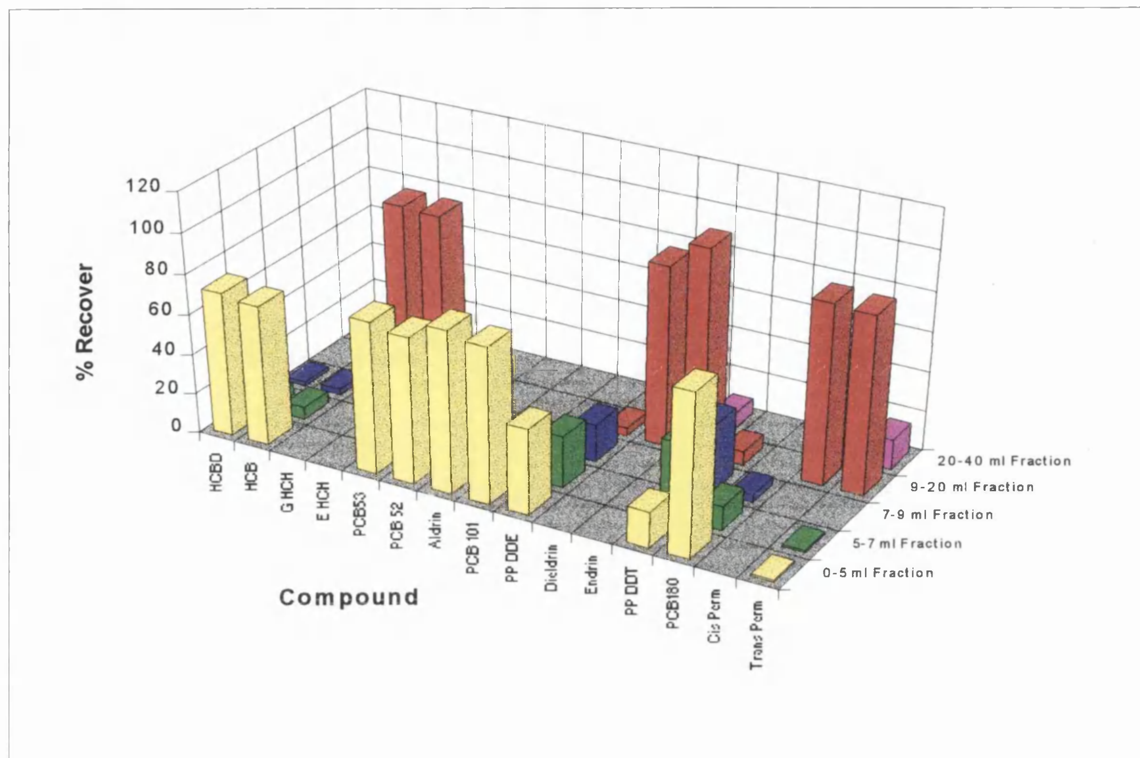
Compound	% Recovery				
	0-5 ml Fraction	5-7 ml Fraction	7-9 ml Fraction	9-20 ml Fraction	20-40 ml Fraction
HCBD	72 (4.6)	6	2	ND	ND
HCB	70 (3.5)	6	3	1	ND
γ HCH	ND	ND	ND	90 (3.9)	5
ε HCH	ND	2	3	89 (4.8)	3
PCB 53	76 (3.9)	12	4	ND	ND
PCB 52	73 (3.1)	17	2	ND	ND
Aldrin	81 (4.1)	23	ND	ND	ND
PCB 101	78 (3.6)	16	6	ND	ND
PP DDE	43 (12.4)	26 (23.8)	18.7 (19.5)	4	ND
Dieldrin	ND	ND	ND	90 (4.6)	5
Endrin	ND	ND	ND	103 (5.3)	8
PP DDT	18	38 (25.4)	34 (17.5)	7	ND
PCB180	81	12	4	ND	ND
Cis Permethrin	ND	ND	ND	90 (3.0)	10
Trans Permethrin	1	1	ND	89 (2.8)	15

ND - Not detected

No RSD presented for recoveries <20%

These results are also shown graphically in Figure7. The results are in excellent agreement with the first test. PCBs, HCBD, HCB and aldrin elute from the column first, followed closely by PP DDE and PP DDT. The OCPs γ HCH, ε HCH, dieldrin, endrin, cis and trans permethrin are eluted by the addition of the 10% MTBE hexane mixture. This test shows that this method cannot separate PP DDE from the PCBs. The results show that by using a cut volume of 9 mls PP DDE and PP DDT could be quantitatively recovered in Fraction 1. Quantification of PP DDT would still be difficult due to co-elution with PCBs.

Figure 7 % Recovery of OCPs and PCBs on a 2g 3.5 % Deactivated Silica Column



The RSD of recoveries of the major portion of the analytes is less than 5% and for PP DDE and PP DDT 20-30%. It was speculated that this poor replication of DDE and DDT may be due to uneven deactivation of the silica gel. It was noted that on addition of the 3.5 mls of water to the silica during preparation the silica stuck together in clumps. These clumps were difficult to break down even after repeated shaking. This suggested that the silica was not a uniform bed of deactivated silica but a mixture of active and deactivated silica. This non uniform reagent might be the source of variation in column retention characteristics.

To remove the possibility of reagent variation the experiment was repeated using only active silica(i.e. silica prepared as described earlier without deactivation by the addition of 3.5% water).

3.4.1.3 Active Silica Gel Column Chromatographic Fractionation of PCBs and OCPs.

Preparation of Reagents

1. Active Silica Gel

   Approximately 100g of silica was heated in a furnace at 500 °C for 4 hours. The silica was cooled in the furnace to 200 +/- 50 °C then transferred to a dessicator where it was cooled to room temperature. The cool silica was transferred to a screw cap bottle and stored in a dessicator until use.

The sodium sulphate, recovery standard, and internal standards were prepared as described previously.

Silica columns were prepared using active silica as described previously, six 1 ml portions of the recovery standard were taken through the analytical procedure described previously. The mean recoveries of each analyte along with their relative standard deviations in brackets are listed in Table 38 and presented graphically in Figure 8.

TABLE 38 % Fractionation of OCPs and PCBs on a 2g non deactivated Silica Column.

Compound	% Recovery				
	0-5 ml Fraction	5-7 ml Fraction	7-9 ml Fraction	9-20 ml Fraction	20-40 ml Fraction
HCBD	77 (3.8)	8	ND	ND	ND
HCB	85 (3.0)	13	ND	ND	ND
γ HCH	ND	ND	ND	16	85 (4.3)
ε HCH	ND	ND	1	20	82 (3.7)
PCB 53	81(3.3)	14	3	ND	ND
PCB 52	86(2.7)	12	1	ND	ND
Aldrin	86(3.6)	11	ND	ND	ND
PCB 101	82(2.8)	13	2	ND	ND
PP DDE	35	41 (5.1)	14	3	ND
Dieldrin	ND	ND	ND	11	89 (2.7)
Endrin	ND	ND	ND	8	93 (1.9)
PP DDT	ND	14	35	43 (6.4)	1
PCB180	88 (1.7)	8	ND	ND	ND
Cis Permethrin	ND	ND	ND	8	97 (1.6)
Trans Permethrin	ND	ND	ND	4	93 (2.3)

ND - Not detected

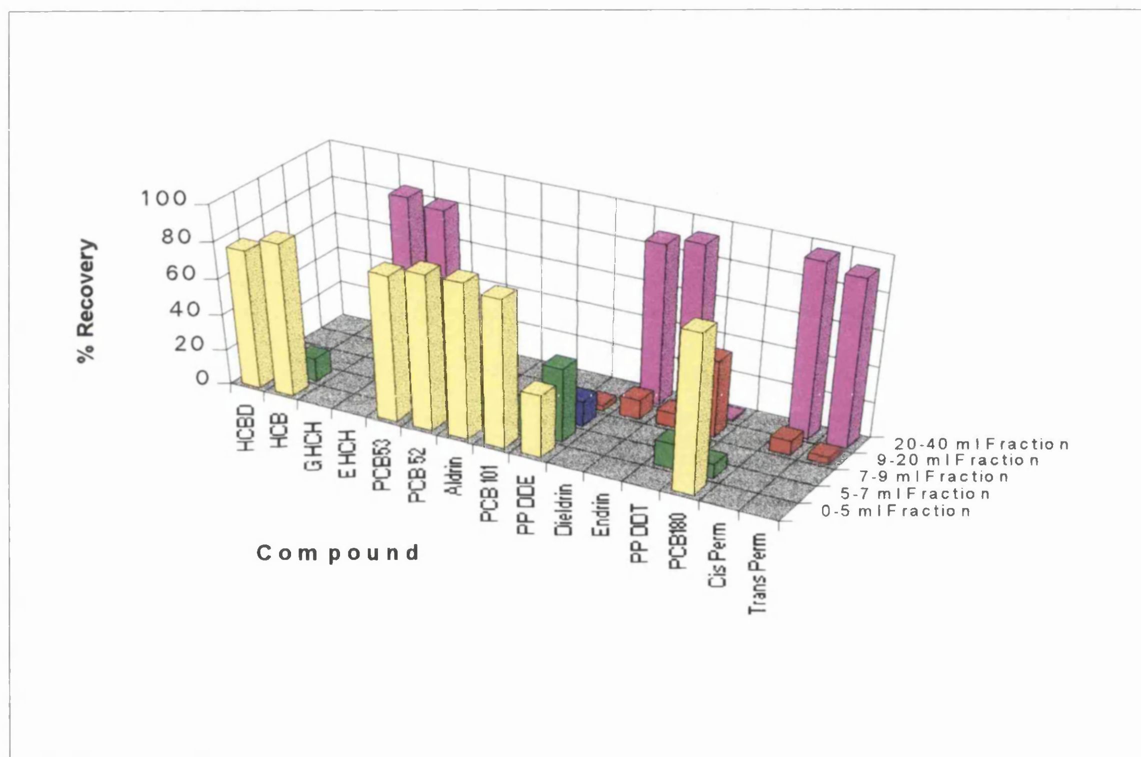
No RSD presented for recoveries <50%

An elution pattern similar to 3.5% deactivated silica is obtained. PCB's, HCBD, HCB and aldrin are eluted first followed closely by PP DDE and PP DDT. The OCPs γ HCH, ε HCH, dieldrin, endrin, cis permethrin and trans permethrin are eluted following addition of the 10 % MTBE/hexane mixture. The elution pattern is the same for both active and deactivated silica, but the pesticides are more strongly retained on the active silica. The active silica column gives a clear separation of PP DDT from the PCB's with 78% PP DDT eluted in the 7-20mls fraction. Poor separation of PP DDE from the PCB's is achieved with 76% of PP DDE eluted in the first 7mls along with the PCBs. This reinforces the finding that it is not possible to separate PP DDE from the PCBs.

The RSD of the PP DDE and PP DDT replicates is improved four fold for the active silica columns. This confirms the theory that uneven deactivation of the silica is a significant source of variation in the procedure.

This experiment clearly demonstrates that active silica provides an improved separation of PCBs from OCPs, and that active silica can quantitatively separate PP DDT from PCB's.

Figure 8 % Recovery of OCPs and PCBs on a 2 g non Deactivated Silica Column



The experiment does not determine the exact volume required to maximise recovery of PP DDE in Fraction 1, while maximising the recovery of PP DDT in Fraction 2. It is indicated that the optimum cut volume is between 7-9 mls.

To optimise the use of active silica separation tests were carried out with the cut volume at 7, 8 and 9 mls. The procedure was repeated three times at each cut volume. The mean recoveries are presented in Table39.

Table 39 Investigation of Optimum Cut Volume on Active Silica Gel Column.

Compound	Test 1 0-7 mls	7-40 mls	Test 2 0-8 mls	8-40 mls	Test 3 0-9mls	9-40mls
HCBD	99	5	96	3	98	2
HCB	100	4	97	4	97	3
γ HCH	ND	107	ND	103	ND	98
ε HCH	ND	104	ND	100	ND	99
PCB 53	92	5	95	3	98	2
PCB 52	94	14	98	6	97	5
Aldrin	98	5	97	2	97	4
PCB 101	98	4	101	2	99	3
PP DDE	78	16	93	7	91	8
Dieldrin	ND	102	ND	102	ND	101
Endrin	ND	106	ND	107	ND	97
PP DDT	11	88	14	89	24	76
PCB 180	96	7	97	5	96	5
Cis Permethrin	ND	100	ND	99	ND	102
Trans Permethrin	ND	100	ND	98	ND	98

The optimum cut volume for active silica is 8 mls.

3.4.1.4 Investigation of Storage of Active Silica Gel.

Previous tests demonstrate the optimum elution volume is influenced by the degree of activation /deactivation of the silica gel. Silica is a strong desiccant and it is suggested that it may become deactivated during storage. Tests were undertaken to determine the effect of storage on the activity of prepared silica.

Active silica gel and sodium sulphate were prepared and stored as described previously. Each day for a period of 5 days a portion of active silica gel and sodium sulphate was removed from the storage jar and 3 optimised replicate recovery tests carried out. The mean recoveries in each fraction for each day are presented in Table 40.

Table 40 Mean % recovery of PCBs and OCPs from an active silica gel column over a 5 day period.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Compound	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
HCBD	94	ND	93	ND	98	ND	95	ND	96	ND
HCB	96	ND	101	ND	94	ND	94	ND	99	ND
γ HCH	ND	100	ND	99	ND	103	ND	99	ND	98
ε HCH	ND	102	ND	98	ND	103	ND	102	ND	104
PCB 53	96	4	98	ND	97	4	96	6	99	2
PCB 52	94	ND	92	ND	93	ND	97	ND	93	ND
Aldrin	97	4	98	ND	100	ND	103	ND	98	3
PCB 101	98	4	96	2	100	ND	99	ND	97	6
PP DDE	88	13	92	9	97	6	91	14	93	11
Dieldrin	ND	104	ND	102	ND	108	ND	103	ND	102
Endrin	ND	102	ND	104	ND	102	ND	99	ND	106
PP DDT	13	88	7	92	9	93	6	86	11	92
PCB180	99	ND	101	ND	98	ND	99	ND	98	ND
Cis Permethrin	ND	103	ND	100	ND	104	ND	100	ND	102
Trans Permethrin	ND	105	ND	101	ND	102	ND	103	ND	98

Prepared silica gel is stable for column use over a 5 day period when stored under conditions outlined above.

3.4.2 Optimisation of Reverse Phase Clean-up using Aminopropyl Bonded Silica Cartridges.

Aminopropyl bonded silica cartridges have been used for the speciation of lipid classes (Simpson, 1992; Kaluzny *et al.*, , 1985). A logical extension of this is the use of aminopropyl bonded silica cartridges for the removal of lipids from the solvent extracts of environmental samples.

Aminopropyl phases take advantage of the differing ability of molecules to hydrogen bond with the bonded phase. Polar molecules such as free fatty acids, monoglycerides, diglycerides and cholesterol interact very strongly with the bonded phase in the presence of a non polar solvent such as hexane. Less polar compounds with no free hydroxyl groups such as triglycerides, phospholipids, and cholesteryl esters interact weakly due to the polarity of the ester linkages. Very non polar compounds such as PCBs do not react with the bonded phase at all.

This suggested that aminopropyl cartridges could be used as a first stage clean-up, to remove large amounts gross interferences.

The aim of this clean-up step and subsequent normal phase clean-up procedures was to obtain quantitative recovery for a wide range of analytes whilst maximising the retention of coextracted interfering compounds.

The Aminopropyl cartridges and the normal phase clean-ups were optimised using the following general experimental design:

1. Optimisation of analyte elution.
2. Optimisation of coextracted material retention.
3. Comparison of different manufacturers products.
4. Investigation of ability to be scaled up to remove very large amounts of coextracted material.

3.4.2.1 Optimisation of Analyte Elution.

1 ml of the analyte recovery test solution prepared previously was taken through the procedure outlined below.

1. Using a 20ml glass syringe Isolute 500mg aminopropyl bonded silica cartridges were activated by passing 10 ± 2mls of methanol through the cartridge at approximately 20 mls/min. Care was taken to ensure no air was pushed through the cartridge.

2. 20 ± 5 mls of hexane was passed through the cartridge at approx. 20 mls/min, care was taken to ensure no air was passed through the cartridge..

3. Using a 1ml glass syringe 1 ± 0.1ml of the recovery standard was passed through the cartridge at 5ml/min, and the eluate collected in a small turbovap tube.

4. Cartridge elution was tested with a number of solvents, see table 41. For each test the eluate was collected in the turbovap tube used in (3).

5. The volume of the extract was reduced to 0.5mls in a turbovap concentrator, using the procedure outlined in section 3.3. The extract was quantitatively transferred to a clean labelled GC vial, internal standards added, the vial sealed and shaken thoroughly.

6. The extract was analysed by GC/ECD using the conditions outlined previously.

Table 41 - Optimisation of Organochlorines Recovery From 500mg AP tubes-Elution solvent

AP Elution Test1	Elute with 10mls Hexane
AP Elution Test 2	Elute with 20mls Hexane
AP Elution Test 3	Elute with 20mls 10% MTBE in Hexane
AP Elution Test 4	Elute with 20mls 20% MTBE in Hexane

Each test was carried out 6 times and the mean % recovery of each analyte was calculated.

The mean % recovery for each analyte is listed below in Table42.

Table 42 Optimisation of Analyte Elution From Aminopropyl Cartridges.

Compound	10mls Hexane	20mls Hexane	20mls 10%MTBE Hexane	20mls 20% MTBE Hexane
HCBD	90	92	90	91
HCB	113	114.5	112	110
γ HCH	117	118	117	118
ε HCH	58	116	113	118
PCB 53	103	98	104	102
PCB 52	113	114	114	112
Aldrin	98	96	100	101
PCB 101	95	98	100	96
PP DDE	90	86	97	100
Dieldrin	100	100	100	100
Endrin	103	96	94	83
PP DDT	76	54	44	39
PCB 180	103	104	104	103
Cis Permethrin	107	108	109	108
Trans Permethrin	105	104	109	108

These results are very encouraging, with greater than 80% recoveries being obtained for most of the compounds, with all tested elution solvents.

ε HCH and PP DDT are the only two compounds giving low recoveries.

PP DDT gives lower recoveries as the strength of the elution solvent increases. This is contrary to the anticipated retention of the aminopropyl phase. Analyte retention should decrease with increasing polarity of the elution solvent. Careful inspection of the chromatograms indicated the presence of PP DDD in the extracts. PP DDD was not present in the original spiking solution. This suggests that PP DDT is not being retained on the aminopropyl column but is either degraded on the aminopropyl column or degraded during the GC/ECD analysis. A standard containing PP DDT was GC/ECD analysed shortly after the above tests and approximately 60% degradation of PP DDT was observed. This confirmed that the degradation was taking place during the GC/ECD analysis, and that PP DDT was being quantitatively recovered from the aminopropyl column.

The average recovery of endrin throughout all the tests was 94%, but , like PP DDT the recovery decreased slightly as the tests progressed . Inspection of the chromatograms from the 20% MTBE test revealed the presence of the endrin degradation products endrin aldehyde and endrin ketone. The presence of these compounds confirms the degradation of endrin during the GC analysis.

Low recoveries of ε HCH were observed when eluting with 10mls of hexane. This was unexpected as ε HCH contains no free OH groups to hydrogen bond with the aminopropyl phase and its isomer γ HCH was quantitatively recovered with 10 mls of hexane.

It seems unlikely that the low recovery of ε HCH is due to hydrogen bonding as higher recoveries were obtained by eluting with a larger volume of hexane. Earlier work has shown that ε HCH is strongly absorbed on silica and it is possible that a secondary interaction between non aminopropyl bonded active sites on the silica and the ε HCH are responsible for its strong retention.

These results clearly demonstrate that a wide variety of analytes can be quantitatively recovered from an aminopropyl column using 20 mls of hexane as the elution solvent. No increases in recoveries were obtained by eluting with a more polar solvent such as 10% MTBE in hexane. Therefore, 20 mls of hexane was selected as the optimum elution conditions for a 500mg Isolute aminopropyl bonded silica cartridge.

3.4.2.2 Investigation of Coextracted material retention.

To enable comparison between different clean-up techniques it was considered necessary to determine the amount of coextracted material each clean-up could remove. This information is required to ensure the clean-up medium was not overloaded allowing coextracted materials to pass through the clean-up to the final determination damaging capillary columns and detectors.

Extracts from the different matrix types contain a wide variety of different co-extracted compounds. No one matrix could be considered as representative of all sample types. To anticipate as wide a range of problems as possible 3 different "test" matrices were prepared and the amount of material adsorbed by the aminopropyl bonded silica column for each type of matrix determined gravimetrically.

Preparation of "Test" Matrices.

Test Matrix 1: Cod liver oil was selected as representative of a high fat content matrix containing high levels of lipids such as triglycerides. This type of extract would be encountered when analysing "Fatty" tissue samples such Eel tissue, and fish livers. This solution was prepared by dissolving 2.5 g of Seven Seas Cod liver oil in 50 mls of hexane. To determine lipid content 3 separate 1ml portions of this extract were added to pre weighed glass vials using a 1ml syringe. These extracts were reduced to dryness in

an oven at 50 °C and then baked to constant weight in an oven at 110°C. Using this technique the mean residue was 47 mg/ml.

Test Matrix 2: Mussels *Mytilus Edulis* were selected as representing low fat tissue of the type encountered with fish muscle or shell fish. A sample homogenate of mussels collected from sites around the Clyde Estuary was freeze dried, ground and mixed to give a uniform powder. This matrix solution was prepared by soxhlet extracting 25 g of the mussel powder with 200mls of 50% MTBE /hexane for 6 hours. The lipid concentration was determined using the gravimetric technique described for test matrix 1 the mean residue was 35mg/ml.

Test Matrix 3: This matrix solution was prepared by soxhlet extracting 100g of freeze dried Clyde Estuary sediment with 200mls of 50% MTBE/hexane for 6 hours. The resulting extract was reduced in volume to 50mls by turbovap concentration. This type of extract was selected as representing those encountered when analysing marine sediments and as worst case scenario for freshwater sediments, sewage effluents and river waters. Using the gravimetric technique described for test matrix 1 the mean residue was 28 mg/ml.

0.5, 1, 2 and 3ml portions of each of the test matrices were passed through 500mg aminopropyl bonded silica cartridges using the optimum conditions described previously. The resulting eluates were collected in Turbovap tubes and their volume reduced to 0.5 mls by turbovap concentration. The resulting concentrates were quantitatively transferred to clean pre-weighed vials and evaporated to constant weight as described for the preparation of test matrices.

These tests were carried out in triplicate, the mean amount of extracted material adsorbed by the Aminopropyl bonded silica cartridge is shown in Tables 43 to 45.

Table 43 Test Matrix 1 (Cod Oil)

	Weight of residue (mg)			
Amount added to Cartridge	23.5	47	94	141
Amount Recovered from Cartridge	15.4	31.6	65.3	113
Amount Retained on Cartridge	8.1	15.4	28.7	28.0

These results indicate the cod oil contains a variety of different lipid types, only a portion (approx. 25%) of the different lipid classes contained in cod oil are retained by the aminopropyl bonded silica cartridge. The cartridge is capable of retaining approximately 28mg of these lipids.

Table 44 Test Matrix 2 (Mussels)

	Weight of residue (mg)			
Amount added to Cartridge	17.5	35	70	105
Amount Recovered from Cartridge	5.1	9.7	38.6	72.4
Amount Retained on Cartridge	12.4	25.3	31.4	32.6

The mussel extract like the cod oil extract contained a variety of different lipid classes. Only a portion of these (approx. 70%) are retained on the cartridge. The cartridge retains approximately 30 mg of these lipids and its performance was not affected by overloading. Before clean-up the mussel extract was highly coloured (Yellow/Orange). After clean-up no colour was observed in the eluates from the 0.5 and 1 ml portions of the mussel extract and a band of orange was observed on the upper portion of the aminopropyl bonded silica cartridges. The eluates from the 2 and 3ml portions of mussel extract were both slightly coloured. The clean-up cartridge used for the 3ml portion of mussel extract was orange over its entire length, indicating that the cartridge had been overloaded. The aminopropyl bonded silica cartridges were very efficient at adsorbing the pigment from mussel extracts.

Table 45 Test Matrix 3 (Clyde Estuary Sediment)

	Weight of residue (mg)			
Amount added to Cartridge	14	28	56	84
Amount Recovered from Cartridge	11.2	21.8	47.6	72.3
Amount Retained on Cartridge	2.8	6.2	8.4	11.7

It was expected that sediment extract would contain only a small amount of lipid. The results confirm this indicating only a small amount of material being adsorbed by the cartridges.

The sediment extract was also highly coloured (green/black) and even after clean-up of the extracts remained highly coloured, however, they were now a dark yellow colour. A tight black band was observed on the upper portion of all the cartridges after the clean-up. Pigments were once again strongly adsorbed by the cartridge.

3.4.2.3 Comparison of Different Manufacturers Aminopropyl Cartridges

Using the optimum conditions determined earlier a blank and 1 ml portions of the recovery standard were cleaned-up using 3 different manufacturers columns (Isolute, Varian, and Baker). The extracts were analysed and the mean % recovery and RSD for each analyte calculated. In addition 3 ml portions of the test matrices were also processed and the amount of material retained on the cartridges calculated. The results for these test are presented below in Table 46.

Table 46 Investigation of Different Manufacturers Aminopropyl Bonded Silica Columns

Manufacturer	ISOLUTE 500mg Cartridge		VARIAN 500mg Cartridge		BAKER 500mg Cartridge	
COMPOUND	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	92.4	3.78	91.6	3.91	90.3	4.16
HCB	101.8	7.86	98.6	4.72	98.5	2.13
γHCH	93.2	4.56	91.6	4.27	92.7	2.71
εHCH	88.0	6.57	85.0	4.86	90.1	3.80
PCB53	100.5	3.10	97.1	3.40	97.6	2.12
PCB52	102.4	3.30	97.3	2.61	100.6	2.87
ALDRIN	98.8	3.80	94.2	5.08	99.1	4.09
PCB101	103.9	4.08	96.3	3.60	98.6	2.54
PP DDE	98.6	5.11	93.6	6.72	98.2	4.23
DIELDRIN	96.1	3.52	93.0	3.70	93.4	3.12
ENDRIN	90.0	4.50	91.4	4.17	91.2	2.89
PPDDT	97.9	3.28	94.8	6.73	95.2	3.16
PCB180	96.9	4.30	92.0	5.11	94.0	2.89
CIS Permethrin	90.9	3.54	91.5	2.25	89.5	4.03
Trans Permethrin	89.4	3.72	91.2	2.21	88.6	3.57
Test Matrix 1 (Cod Oil)	28.4		25.1		28.1	
Test Matrix 2 (Mussel Extract)	31.8		28.5		33.1	
Test Matrix 3 (Sediment)	11.5		10.1		11.8	

The tested manufacturers cartridges performed similarly. The Varian cartridge retained approximately 10 % less coextracted material than either the Isolute or Baker cartridges. All the cartridges were priced similarly, therefore, the Varian cartridges were not considered suitable for routine use.

3.4.2.4 Investigation of Ability to be Scaled Up

To investigate possible correlations of levels of contaminants with sub lethal biological effects the environmental analyst is continually required to push the detection limits of techniques lower. If very large amounts (>300 mg) of coextracted material could be removed from extracts it would allow the analysis of larger amounts of sample and hence improve the detection limits.

Custom manufactured Isolute 10g Aminopropyl Cartridges were obtained on a free trial basis from Crawford Scientific. The use of these 'Jumbo' cartridges for the removal of large amounts of coextracted material was investigated.

Direct scaling up of the optimised procedure indicated these cartridges would require elution with 400mls of hexane to obtain quantitative recoveries of the analytes. Due to the length of time required to evaporate large volumes and the potential for introducing interferences 400mls of hexane was not considered a practical method of eluting the cartridges. 1ml portions of the analyte recovery solution were processed as per previous tests using a variety of elution solvents. The % recovery of these analytes with the different elution solvents are listed in Table 47.

Table 47 Elution of Analytes from a 10g Aminopropyl Cartridge.

COMPOUND	30mls Hexane	60mls Hexane	90mls Hexane	120mls Hexane	150mls Hexane	30mls 1% MTBE in Hexane	30 mls 5% MTBE in Hexane	30mls 10% MTBE in Hexane
HCBD	76	81	93	101	95	77	73	75
HCB	88	94	103	97	105	93	81	87
γ HCH	34	52	69	81	90	41	52	73
ε HCH	0	1	32	57	61	0.0	1	3
PCB53	91	95	90	93	91	96	67	71
PCB52	90	94	102	107	104	95	73	76
ALDRIN	89	92	99	99	98	92	78	83
PCB101	87	89	84	87	84	90	89	94
PP DDE	85	88	87	87	86	89	87	89
DIELDRIN	87	84	87	93	90	86	90	88
ENDRIN	94	97	100	99	97	96	102	101
PPDDT	69	71	70	74	74	62	64	69
PCB180	91	94	96	90	91	94	94	96
Cis Permethrin	85	82	87	89	80	80	83	87
Trans Permethrin	83	83	86	88	89	80	88	89

Acceptable recoveries are obtained for all of the test analytes except PP DDT, γ HCH and ε HCH. 150mls of hexane is required to obtain acceptable recoveries of γ HCH. None of the tested elution solvents gave quantitative recoveries for ε HCH. As in previous tests the low recoveries of ε HCH cannot be explained by the behaviour of the bonded phase suggesting that secondary interactions are responsible for the retention of ε HCH. The large elution volume required for γ HCH suggests that γ HCH is also retained by secondary interactions. These columns are not suitable for the clean-up of samples in which isomers of HCH were target analytes. The large adsorbent bed mass of these columns made their use difficult, a large amount of force was required to push the elution solvent

through the column. These columns were not considered suitable for the routine clean-up of large amounts of coextracted material.

3.4.3 Investigation of Normal Phase Clean-up using Florisil Cartridges.

Florisil is powdered magnesia - silica gel, and is a general purpose polar adsorbent which has been widely used in manually prepared adsorption columns for the clean-up of pesticide residue extracts. Recently florisil has become available in manufactured disposable cartridges, these cartridges are purchased in sealed packs of 6 and are ready for use on breaking the seal of the pack. This makes them considerably quicker, easier and more convenient to use than traditional manually prepared clean-up columns. In addition the manufacturers quality specification tests ensure a product of consistent quality. Isolute florisil PR grade cartridges were selected as cartridges suitable for the clean-up of environmental extracts. The following test were carried out to optimise and evaluate their performance.

3.4.3.1 Optimisation of Analyte Elution

1 ml of the analyte recovery solution as used before was taken through the procedure outlined below.

1. Using a 20ml glass syringe Isolute 500mg florisil cartridges were washed by passing 10 ± 2ml of hexane through the cartridge at approximately 20 mls/min. Care was taken to ensure no air was pushed through the cartridge.

2. Using a 1ml glass syringe 1 ± 0.1ml of the recovery standard was passed through the cartridge at 5ml/min, and the eluate collected in a small turbovap tube.

3. The cartridge was eluted with a variety of solvents given in Table 48. The eluate for each solvent was collected in the turbovap tube used in (2).

4. The volume of the eluate was reduced to 0.5mls according to optimised turbovap conditions found in section 3.3. The reduced solution was quantitatively transferred to a GC vial, Internal standards were added and the vial was sealed and shaken thoroughly.

5. The extracts were analysed by GC/ECD using the optimised conditions determined in section 3.2 and the % recovery of each analyte determined.

Table 48 - Elution solvents for the Optimisation of analyte recovery from 500mg Florisil Cartridges

Test No.	Elution Solvent
Florisil Test1	10mls Hexane
Florisil Test 2	20mls Hexane
Florisil Test 3	20mls 10% MTBE in Hexane
Florisil Test 4	20mls 20% MTBE in Hexane

Each test was replicated 3 times. The mean % recovery and RSD for each analyte are listed in Table 49.

Table 49 % Recovery of PCBs and OCPs from a 500mg Isolute Florisil cartridge.

Elution solvent	10 mls Hexane		20 mls Hexane		20 mls 10% MTBE in Hexane		20 mls 20% MTBE in Hexane	
Compound	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	85	3.1	83	0.6	78	1.4	107	3.5
HCB	102	1.2	110	4.3	113	1.2	105	4.1
γ HCH	82	1.4	114	0.8	104	2.6	103	3.4
ε HCH	64	4.6	106	4.4	102	3.8	102	0.7
PCB 53	102	4.1	106	3.7	99	2.8	103	2.6
PCB 52	102	2.0	110	1.9	104	2.5	105	2.1
Aldrin	98	3.2	101	3.6	103	4.1	102	3.3
PCB 101	101	4.5	98	3.2	103	3.4	104	3.1
PP DDE	106	2.6	104	3.2	96	0.5	102	2.2
Dieldrin	ND		47	10.3	101	0.2	103	0.9
Endrin	12	12.2	55	9.9	104	0.7	103	2.1
PP DDT	14	11.2	36	16.2	105	1.3	108	0.6
PCB 180	95	1.6	101	13.8	106	1.1	105	0.9
Cis Permethrin	ND		2	35.0	102	0.4	102	1.8
Trans Permethrin	ND		ND		103	0.8	105	2.4

Elution with 10 mls of hexane gives quantitative recoveries for PCBs, HCB, HCBD and aldrin. Low recoveries are obtained for most of the OCPs and no recovery of dieldrin, endrin, cis and trans permethrin.

Elution with 20mls of hexane gives an improvement in the recovery for most of the OCPs. The recoveries are unacceptable for dieldrin, endrin, cis and trans permethrin, with only approximately 50 % recoveries of dieldrin and endrin, and virtually no recovery for permethrins. Elution with 20 mls of 10 % MTBE in hexane significantly improves the recoveries of the OCPs. Recoveries are acceptable for all of the test analytes. Elution with 20 mls of 20 % MTBE in hexane gives acceptable recoveries for all of the test analytes. No difference is observed in the recovery of the test analytes when using 10 % and 20 % MTBE in hexane. 10 % MTBE in hexane was selected as the optimum elution solvent as a higher concentration of MTBE could possibly increase the elution of coextracted materials from the column when used with sample extracts.

Further Optimisation of Analyte Elution.

The above results suggest that the strength of the elution solvent increases with increasing MTBE concentration. In order to ensure maximum retention of coextracted compounds on the florisil cartridge the above tests were repeated using 20 mls of 2 % MTBE in hexane and 5% MTBE in hexane. The results of these tests are presented below in Table 50.

Table 50 % Recovery of PCBs and OCPs from a 500mg Isolute Florisil Cartridge

Elution Solvent	20 mls 2 % MTBE in Hexane		20 mls 5 % MTBE in Hexane	
Compound	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	91	3.1	94	4.1
HCB	103	4.7	105	2.4
γ HCH	102	1.0	100	0.8
ε HCH	104	1.2	99	1.3
PCB 53	103	2.4	102	3.1
PCB 52	106	2.1	103	1.6
Aldrin	106	1.4	103	1.1
PCB 101	103	2.4	102	2.1
PP DDE	104	2.0	101	1.0
Dieldrin	67	3.89	96	1.4
Endrin	61	2.8	93	2.9
PP DDT	41	4.1	87	3.2
PCB 180	103	1.3	105	1.3
Cis Permethrin	4	-	46	4.2
Trans Permethrin	3	-	51	3.6

Elution of the column with 20 mls of 2 % MTBE in hexane gives quantitative recoveries for PCBs, HCB, HCBD, and aldrin. Low recoveries are obtained for most of the OCPs and almost no recovery of cis and trans permethrin.

Elution of the column with 20 mls of 5 % MTBE in hexane gives acceptable recoveries for all of the analytes except cis and trans permethrin. The recoveries of cis and trans permethrin are improved by an order of magnitude by the increase in polarity of the elution solvent, but at 50% are still unacceptably low. Elution with 20mls of 10 % MTBE in hexane was selected as the optimum conditions.

3.4.3.2 Investigation of Coextracted Material Retention.

The retention of coextracted material was investigated using the test matrices prepared in section 3.4.2.2.

0.5, 1, 2 and 3ml portions of each of the test matrcies were passed through 500mg florisil cartridges using the optimum conditions determined previously. The resulting eluates were collected in turbovap tubes and their volume reduced to 0.5 mls by turbovap concentration. The resulting extracts were quantitatively transferred to clean pre-weighed vials and evaporated to constant weight as described for the preparation of test matrices.

These tests were carried out in triplicate. The mean amount of extracted material adsorbed by the florisil cartridge is shown in Tables 51 to 53.

Table 51 Test Matrix 1 (Cod Oil)

	Mean Weight of residue (mg)			
On cartridge	23.5	47	94	141
Recovered	0.4	18.4	65.3	112.8
Retained	23.1	28.6	28.7	28.2

A 500mg florisil column eluted with 20mls of 10 % MTBE in hexane retains a maximum of approximately 28 mg of cod oil type lipid material. In contrast to the reverse phase aminoproyl bonded silica clean-up the florisil cartridge is capable of retaining the majority of lipid types from the cod liver extract.

Table 52 Test Matrix 2 (Mussels)

	Mean Weight of residue (mg)			
On cartridge	17.5	35	70	105
Recovered	1.2	7.7	40.9	75.5
Retained	16.3	27.3	29.1	29.5

A 500mg florisil column eluted with 20mls of 10 % MTBE in hexane retains approximately 29 mg of Mussel lipids material. These extracts were highly coloured and the florisil cartridge did remove a portion of the colour from the extract, however, the 17.5 mg (0.5 mls) residue extract remained slightly coloured after passing through the cartridge and the other extracts all remained highly coloured.

Table 53 Test Matrix 3 (Sediments)

	Mean Weight of residue (mg)			
On cartridge	14	28	56	84
Recovered	0.2	4.0	28.6	55.7
Retained	13.8	24.0	27.4	28.3

A 500mg florisil column eluted with 20mls of 10 % MTBE in hexane retains a maximum of approximately 25 mg of extractable material from Clyde Estuary sediments. The extracts were highly coloured the florisil cartridge did remove a portion of the colour from the extract. All the extracts remained highly coloured after passing through the cartridge.

3.4.3.3 Comparison of Different Manufacturers Florisil Adsorption Cartridges

Using the optimum conditions determined earlier a blank and 1 ml portions of the recovery test solution were cleaned-up using 3 different manufacturers columns (Isolute, Varian, and Baker). Internal Standards were added and the extracts were analysed by GC/ECD analysis. The mean % recovery and RSD for each analyte were calculated. In addition 3 ml portions of the test matrices were processed and the amount of material retained on the cartridges calculated. The results for these tests are presented below in Table 54.

Table 54 Comparison of Different Manufacturers Florisil Cartridges

Manufacturer	ISOLUTE 500mg Cartridge		VARIAN 500mg Cartridge		BAKER 500mg Cartridge	
COMPOUND	Mean % Recovery	RSD	Mean % Recovery	RSD	Mean % Recovery	RSD
HCBD	91.1	3.5	78.6	4.6	92.3	3.2
HCB	98.6	2.1	82.7	4.1	98.5	3.1
γ HCH	101.3	3.2	87.6	3.8	103.2	2.7
ε HCH	103.2	4.1	91.3	4.0	100.2	2.0
pcb53	99.4	3.6	92.3	3.4	99.4	3.2
pcb52	100.8	3.0	88.0	2.7	97.5	2.8
aldrin	99.6	2.8	89.9	2.8	99.3	3.0
pcb101	99.0	2.8	91.2	3.2	100.3	3.1
pp DDE	98.1	3.2	91.2	3.4	99.1	2.7
dieldrin	100.2	2.6	90.1	3.5	101.4	3.4
endrin	98.4	3.7	89.3	4.0	99.8	2.7
pp DDT	97.6	2.9	91.5	2.9	97.9	2.5
pcb180	99.3	3.4	88.2	3.4	98.3	3.2
cis permethrin	97.5	3.2	86.4	3.8	101.3	3.0
trans permethrin	96.4	3.3	88.6	3.6	100.5	3.7
Mean	98.7	3.16	88.46	3.55	99.27	2.95
Test Matrix 1 (Cod Oil)	28.4		25.1		28.1	
Test Matrix 2 (Mussel Extract)	28.8		24.8		29.1	
Test Matrix 3 (Sediment)	28.9		24.6		29.2	

The Isolute and Baker cartridges demonstrate similar performance, with mean analyte recoveries of 98.7 and 99.3 % and a mean coextracted retention of 28.7 and 28.8 mg. The Varian cartridges exhibit a poorer performance. The chromatograms obtained using these cartridges were of a poor quality with a large number of peaks observed in the early portion of the chromatogram (i.e. between HCBD and HCB) in both the blanks and recovery test samples. The mean analyte recovery (88.5 %) and mean coextracted material retention (24.8 mg) are lower than those obtained using the Isolute or Baker cartridges. The Isolute cartridges were selected for use in all subsequent tests.

3.4.4 Optimisation of Clean-up using Alumina as an Adsorbent.

Prior to use in multi-residue clean-up techniques commercial alumnia is routinely pre-treated by a number of different methods. These methods include activation/deactivation, acid/base washing and impregnation with compounds such as silver nitrate and sodium sulphite. The aim of the following series of tests was to investigate the preparation of alumina adsorbents to establish a reliable method for the preparation of an adsorbent of consistent quality for the removal of polar contaminants from extracts of environmental samples.

3.4.4.1 Investigation of Acid / Basic Alumina Column Clean-up.

The MEWAM Nos. 13 and 82 recommend the use of a mixed 3 g Acidic/Basic alumina adsorption column for the cleanup of extracts for multi-residue analysis. This was considered a useful starting point in the investigation of alumina as an absorbent

1ml of the analyte process recovery solution prepared as described previously was taken through the experimental procedure outlined below in triplicate. In addition 3 ml portions of the test matrices described previously were also taken through the experimental procedure. The % recovery of the target analytes were determined by GC/ECD analysis as described in section 3.2. The clean-up capacity was determined by gravimetric analysis of the test matrix extracts as described previously.

Preparation of Reagents

Acidic Alumina :100 +/- 5 g of alumina was mixed with 200 mls of 1M HCL until a slurry was formed. Using a vacuum pump and a Buchner funnel the alumina was collected by filtration. The alumina was transferred to a crucible and heated in a furnace at 500 °C for 4 hours . The alumina was cooled to approximately 200 °C in the furnace and transferred to a desiccator where it was cooled to room temperature. 7g of MiliQ organic free water was added to 100g of alumina in a screw cap conical flask and the contents thoroughly shaken by hand for at least 5mins to produce 7% deactivated acid alumina. This alumina was stored in a sealed glass container in a desiccator and used within 5 days of preparation.

Preparation of alkaline alumina: 100 +/- 5 g of alumnia was mixed with 200 mls of 1M KOH until a slurry was formed. Using a vacuum pump and a Buchner funnel the alumina was collected by filtration. The alumina was transferred to a crucible and heated in a furnace at 500 °C for 4 hours . The alumina was cooled to approximately 200 °C in the furnace and transferred to a desiccator where it was cooled to room temperature. 7g of MiliQ organic free water was added to 100g of alumina in a screw cap conical flask and the contents thoroughly shaken by hand for at least 5mins to produce a 7% deactivated basic alumina. This alumina was stored in a sealed glass container in a desiccator and used within 5 days of preparation

Preparation of Sodium Sulphate: 100 +/- 5 g of Sodium Sulphate was placed in a crucible and heated in a furnace at 500 °C for 4 hours. The sodium sulphate was cooled to approximately 200 °C in the furnace and transferred to a desiccator where it was cooled to room temperature. The sodium sulphate was transferred to a sealable glass container and stored in a desiccator until use. This reagent was used within five days of preparation.

Experimental Procedure

1. A 10mm ID and 50 cm long glass column fitted with a PTFE stopcock was plugged with a small portion of glass wool.

2. Using a small weighing boat and a top pan balance 2g of acidic and 1 g of basic alumina were added to the column. The column was plugged with approx. 5mm of sodium sulphate and tapped vigorously to settle the contents. The column was used immediately after preparation.

3. Using a 1ml syringe, 1ml of the test solution was added carefully to the top of the column. The meniscus was allowed to fall to the sodium sulphate layer and then 35mls of 10% MTBE in hexane was carefully added to the column.

4. The eluate 35mls was collected in a Turbovap tube. This was concentrated to 0.5mls using the Turbovap concentrator using the conditions described in section 3.3.

5. The extract was quantitatively made up to 1ml with iso-octane, internal standards added and taken for either gravimetric or GC analysis.

The mean % recovery and RSD of the target analytes for the 3 determinations are shown in Table 54. The capacity of the column for each of the test matrices is also shown in Table 54.

All the target analytes are quantitatively recovered from the column. The clean-up capacity of the column was less than a 500mg florisil cartridge for test matrix 1 (Cod Oil), and Test matrix 2 (Mussels). The performance was similar to a 500mg florisil cartridge for Test Matrix 3.
While performing this analysis it was observed that the colour in the test matrices particularly matrix 3 (Sediment) was strongly retained on the adsorbent but removed when the MTBE/hexane eluting solvent was applied to the column. Experiments were undertaken to improve retention of the co-extractants by use of a less polar elution solvent

Table 54: % Recovery of PCBs and OCPs From a Mixed Acid/Base Alumina Column.

Compound	Mean % Recovery	RSD
HCBD	94	6.4
HCB	97	8.4
γ HCH	92	5.3
ε HCH	94	3.5
PCB 53	91	7.1
PCB 52	96	4.0
Aldrin	87	3.9
PCB 101	103	7.3
PP DDE	98	4.8
Dieldrin	96	5.8
Endrin	103	4.9
PP DDT	106	5.2
PCB 180	97	4.4
Cis Permethrin	95	6.7
Trans Permethrin	93	7.3
Amount (mg) of Test Matrix 1 (Cod Oil) retained on column	20.9	
Amount (mg) of Test Matrix 2 (Mussel) retained on column	14.4	
Amount (mg) of Test Matrix 1 (Sediment) retained on column	9.4	

Optimisation of Acidic / Basic Alumina Column Clean-up Elution Solvent.

As per the previous procedures the elution solvent polarity was tested to determine the minimum eluate strength required to obtain acceptable recoveries for target analytes with retention on the column of co-extractants. Recovery solutions and test matrices were as per previous investigations. The eluants tested were 100mls of hexane, 35mls of 1% MTBE in hexane and 35mls of 5% MTBE in hexane. As in the previous experiment the % recovery of the target analytes was determined by GC analysis and the clean-up capacity determined by gravimetric analysis of the test matrix extracts.

Mean recoveries of the target analytes and amount of coextracted material retained are listed in Table 55.

Reducing the strength of the eluting solvent significantly increases the retention of the co-extractants. Recoveries of the test analytes were reduced to unacceptable levels. The performance of the mixed acid/base alumina columns is unacceptable. It was anticipated that the acid treated alumina would strongly adsorb basic compounds and basic treated alumina strongly adsorb acidic sample components, resulting in a clean extract with all coextracted compounds removed. However, these tests indicated that the majority of co-extracted compounds from the sample matrices are not strongly attracted to acidic or basic adsorbents. Therefore it was speculated that these compounds must be essentially neutral and hence would be best adsorbed by a neutral alumina.

Table 55 % Recovery of Organochlorine Compounds From Alumina Column Consisting of 2g of 7% Deactivated Acidic Alumina Overlain with 1g 7 % Deactivated Alkali Alumina.

	% Recovery		
Compound	100 mls Hexane	35 mls of 1% MTBE in Hexane	35 mls of 5% MTBE in Hexane
HCBD	84	88	92
HCB	97	101	103
γ HCH	52	53	52
ε HCH	2	7	13
PCB 53	95	102	102
PCB 52	95	103	104
Aldrin	83	103	103
PCB 101	95	101	102
PP DDE	94	98	97
Dieldrin	91	81	93
Endrin	86	79	83
PP DDT	93	92	93
PCB 180	91	104	101
Cis Permethrin	24	51	68
Trans Permethrin	4	5	52
DCBP	101	103	103
Amount (mg) of Test Matrix 1 (Cod Oil) retained on column	26.1	24.9	22.3
Amount (mg) of Test Matrix 2 (Mussel) retained on column	18.2	18.3	16.8
Amount (mg) of Test Matrix 1 (Sediment) retained on column	14.6	14.4	13.0

3.4.4.5 Investigation of Neutral Alumina as an adsorbent for the Clean-up of Environmental Extracts.

Previous experience with Silica Gel had shown that the preparation of deactivated adsorbents was a difficult procedure which if not carried out with great care could lead to significant variability in the elution patterns of the target analytes. Therefore, it was considered desirable to initially investigate use of active alumina. From the Acid/Base alumina column tests it was clear that 3 g was a relatively small amount of adsorbent with a limited adsorption capacity . Therefore, larger columns were used in an attempt to improve the clean-up efficiency.

Preparation of Reagents

Preparation of Neutral alumina: Approximately 200 g of alumina was heated in a furnace at 500°C for 4 hours. The alumina was cooled in the furnace to approximately 200 °C then transferred to a desiccator where it was cooled to room temperature. The alumina was stored in the desiccator prior to use. The alumina was used within 5 days of preparation.

All other reagents were prepared as described previously.

6 g Neutral alumina columns were prepared in a manner similar to previous tests. 1ml portions of the analyte recovery test solution and 3ml portions of the test matrices were cleaned-up using a protocol similar to section 3.4.4.1 with the following elution solvents: 100mls hexane, 100 mls 5 % MTBE in hexane, 100 mls 10 % MTBE in hexane.

As in the previous tests the % recovery of the target analytes was determined by GC analysis, and the retention of the test matrices by gravimetric analysis. These results are presented in Table 56

Table 56 Optimisation of Neutral Alumina columns for the clean-up of environmental extracts.

	100 mls Hexane	100 mls 5 % MTBE Hexane	100 mls 10 % MTBE Hexane
HCBD	74	77	79
HCB	66	71	74
γ HCH	10	11	18
ε HCH	4	6	5
PCB 53	57	55	73
PCB 52	63	71	75
Aldrin	29	40	43
PCB 101	75	69	76
PP DDE	73	72	78
Dieldrin	31	38	44
Endrin	29	43	46
PP DDT	47	65	68
PCB 180	70	76	77
Cis Permethrin	16	20	21
Trans Permethrin	19	27	29
Amount (mg) of Test Matrix 1 (Cod Oil) retained on column	136	121	98
Amount (mg) of Test Matrix 2 (Mussel) retained on column	102	87	64
Amount (mg) of Test Matrix 1 (Sediment) retained on column	61	45	33

6g alumina columns give excellent adsorption of the test matrix co-extractants, with an approximately 3-4 fold increase in adsorption compared to the 500mg florisil cartridges. For all solvents used the recoveries of the test analytes are unacceptable. To obtain quantitative recoveries of the test analytes it is likely the polar strength of the elution solvent would have to be increased or the activity of the adsorbent would have to be reduced.

Previous experience with Acid/Base alumina clean-ups (Test 13) had shown that increasing the % of the polar solvent in the eluent greatly reduced the absorbent capacity of the column. This is confirmed in these tests with between a 30 and 50 % drop in adsorption capacity being observed on increasing the eluent strength from hexane to 10% MTBE in hexane. Deactivation of the alumina by the addition of water was considered as an alternative to increasing the strength of the eluent.

Investigation of % moisture deactivation on Neutral Alumina.

As indicated in section 2.3.1.1 the activity of Alumina (and other normal phase adsorbents) can be varied by adjusting the amount of free hydroxyl groups present on the adsorbents surface. Addition of water to the adsorbent is a common method of increasing the amount of free hydroxyls and hence altering the activity of the adsorbent.

Neutral alumina adsorption columns of increasing deactivation were prepared as described below. Duplicate 1ml portions of the analyte process recovery standard, and 3 ml portions of the test matrices were "cleaned - up" using the experimental procedure outlined previously. 100 mls of hexane was used as the eluate, as in the previous experiments the % recovery of the target analytes was determined by GC/ECD analysis and the adsorption of the test matrices by gravimetric analysis.

Preparation of Reagents

Preparation of 5% Deactivated Neutral alumina: Approximately 200 g of alumina was heated in a furnace at 500°C for 4 hours. The alumina was cooled in the furnace to approximately 200 °C then transferred to a desiccator and cooled to room temperature. The alumina was stored in the desiccator prior to use. The alumina was stored for no more than 5 days prior to use.

10 and 15 % deactivated neutral alumina were prepared in a similar manner. The experimental procedure was similar to the procedure outlined previously.

The mean percentage recoveries of the test analytes and retention of the test matrices are listed in Table 57.

The active, 5 % and 10 % deactivated columns demonstrate excellent retention of the test matrices. The 15 % deactivated columns co-extractant retention is similar to that of the 500mg florisil cartridge.

As indicated earlier the Mussel and Sediment extracts (Test Matrixs 2 and 3) were highly coloured. The active, 5 % and 10 % deactivated alumina columns removed the majority of the colour from the mussel extracts with only a faint yellow tinge observed in the extracts after clean-up. A distinct yellow colour was observed in the mussel extracts after clean-up with 15% deactivated alumina. The active alumina changed the colour of the sediment extract from green/black to yellow, all of the deactivated aluminas changed the colour of the sediment extracts to orange/yellow. All of the sediment extracts remained highly coloured after clean-up.

Table 57 Effect of Water on the Activity of Neutral Alumina.

	Active Neutral Alumina	5 % H_2O Deactivated Alumina	10 % H_2O Deactivated Alumina	15 % H_2O Deactivated Alumina
HCBD	74	83	98	97
HCB	66	98	99	101
γ HCH	10	48	101	103
ε HCH	4	23	96	98
PCB 53	57	98	97	97
PCB 52	63	101	103	100
Aldrin	29	87	99	98
PCB 101	75	104	101	98
PP DDE	73	93	98	96
Dieldrin	31	76	101	101
Endrin	29	73	94	96
PP DDT	47	89	102	103
PCB 180	70	100	100	101
Cis Permethrin	16	44	99	100
Trans Permethrin	19	51	101	103
Amount (mg) of Test Matrix 1 (Cod Oil) retained on column	136	102	94	34
Amount (mg) of Test Matrix 2 (Mussel) retained on column	102	89	80	24
Amount (mg) of Test Matrix 1 (Sediment) retained on column	61	41	39	16

The HCHs and permethrins are the most strongly retained analytes and acceptable recoveries were obtained only for the 10 and 15 % deactivated columns.

The 10 % deactivated Neutral alumina column is the most effective clean-up column investigated. The capacity of the column has a limit of approximately 90mg of lipid from biota matrices and only 40mg of coextracted compounds from sediment extracts.

nvestigation of Temperature on Neutral Alumina Activation.

As indicated in Section 2.3.1.1 a literature search on the uses of alumina as an adsorbent had suggested that the temperature of activation of the alumina greatly influenced the crystal structure of the adsorbent. A series of experiments was carried out to determine whether the furnace activation of Alumina was necessary and if the firing temperature altered the activity of alumina.

Neutral Alumina with no activation, activation by heating in a furnace at increasing temperatures (200, 350, 500, 800 ^{o}C) for 5 hours followed by deactivation by the addition of 10 % water were used in 6g columns as per previous tests.

Preparation of Reagents

Preparation of 10 % Water Deactivated Neutral alumina: Approximately 200 g of alumina was heated in a furnace at 500°C for 4 hours. The alumina was cooled in the furnace to 200 +/- 50 °C then transferred to a desiccator where it was cooled to room temperature. The alumina was stored in the desiccate prior to use.

Preparation of 200 C fired 10 % Water Deactivated Neutral alumina: Approximately 200 g of alumina was heated in a furnace at 200°C for 5 hours. The alumina was cooled in the furnace and then transferred to a desiccator where it was cooled to room temperature. The alumina was stored in the desiccate prior to deactivation. 20g of MiliQ organic free water was added to 200g of alumina in a screw cap conical flask and the contents thoroughly shaken by hand for at least 5mins to produce a 10% water deactivated alumina. This alumina was stored in a sealed glass container in a desiccator and was used within 5 days of preparation

10% water deactivated neutral alumina which had been fired at 350, 500, and 800 °C were also prepared in a manner similar to that described above.

The experimental procedure was similar to the procedure outlined in previous tests. The mean Recovery of the target analytes is listed in Table 58.

Table 58 Effect of Activation Temperature on the activity of Neutral Alumina

	No Heat	200 °C	350 °C	500 °C	800 °C
HCBD	84	84	81	90	90
HCB	97	94	96	97	92
γ HCH	104	96	95	102	105
ε HCH	101	98	95	97	95
PCB 53	107	99	96	96	88
PCB 52	102	90	98	94	95
Aldrin	102	96	97	101	104
PCB 101	101	95	98	96	103
PP DDE	109	97	94	98	101
Dieldrin	102	96	100	99	97
Endrin	115	102	104	105	92
PP DDT	104	102	103	105	98
PCB 180	105	98	98	94	104
Cis Permethrin	108	100	99	91	97
Trans Permethrin	101	102	100	99	97
Mean Recovery	102.8	95.93	96.93	97.6	97.2
Amount (mg) of Test Matrix 1 (Cod Oil) retained on column	24	65	78	94	91
Amount (mg) of Test Matrix 2 (Mussel) retained on column	17	48	65	80	83
Amount (mg) of Test Matrix 1 (Sediment) retained on column	12	27	32	39	41

The recoveries of the target analytes are acceptable for all of the activation temperatures. The alumina which had not been activated produces chromatograms of poor quality with a large number of interfering peaks particularly at the start of the chromatogram. The activation temperature effects the retention of the test matrices on the column. The retention of coextracted material increases with increasing activation temperature. No significant improvement in coextracted retention is observed by increasing the activation temperature from 500 to 800 °C.
500°C was selected as the optimum activation temperature.

3.4.4.3 Chemically Modified Alumina

As indicated in section 2.3.1.1 the activity of adsorbents such as alumina can be altered by the impregnating the surface of the alumina with a chemical reactive towards particular classes of chemicals. These classes of chemicals are then preferentially adsorbed by the absorbent. Silver impregnated alumina has been used (MEWAM No. 13) to increase the adsorption of coextracted interferences from environmental extracts. It has been reported (Snyder, 1968) that silver impregnated alumina exhibits increased retention of compounds such as triglycerides, cholesteryl esters, hydrocarbons, aldehydes and sulphur containing compounds. The use of silver nitrate impregnated columns was investigated.

6 g silver nitrate impregnated alumina columns were prepared as described below , as per previous tests 1 ml portions of the recovery test solutions and 3ml portion of the test matrices were analysed.

Preparation of Reagents

Alumina-silver nitrate: Approximately 200 g of alumina was heated in a furnace at 500°C for 4 hours. The alumina was cooled in the furnace and then transferred to a desiccator where it was cooled to room temperature. 15 g of silver nitrate was dissolved in 20 mls of deionised water, this 75 % w/v silver nitrate solution was added to the 200g of alumina in a screw cap conical flask and the contents thoroughly shaken by hand for at least 5mins to produce a 10% water deactivated 7.5% silver nitrate coated alumina. This silver nitrate alumina was stored in a sealed amber glass container in a desiccator and was used within 4 hours of preparation. Silver Nitrate is corrosive to the skin, gloves were worn at all times when handling solution or solids containing silver nitrate.

Mean recoveries of the target analytes are listed in Table 58.

The silver nitrate impregnated column performs extremely well, quantitative recoveries are obtained for all of the test analytes. The test matrices are all retained to a high degree with no colour observed in any of the eluted extracts

Table 58 Mean Recoveries from Silver Nitrate Impregnated Alumina

Compound	Mean % Recovery
HCBD	88
HCB	94
γ HCH	91
ε HCH	89
PCB 53	96
PCB 52	98
Aldrin	95
PCB 101	98
PP DDE	102
Dieldrin	95
Endrin	94
PP DDT	94
PCB 180	99
Cis Permethrin	88
Trans Permethrin	92
Amount (mg) of Test Matrix 1 (Cod Oil) retained on column	103
Amount (mg) of Test Matrix 2 (Mussel) retained on column	85
Amount (mg) of Test Matrix 1 (Sediment) retained on column	74

3.4.5 Conclusions

Glass columns (500mm * 10mm) containing 2 g of Silica Gel which had been activated by heating in a furnace at 500 °C for 4 hours are suitable for the separation of the majority of OCPs from PCBs. The optimum elution solvents are 10mls of hexane followed by 25 mls of 20% MTBE in hexane. The optimum fractionation of PCBs from OCPs is obtained using a cut volume of 8 mls. When stored in a dessicator the active silica gel is found to be stable for at least 5 days.
Water deactivated Silica Gel shows a wide variation in column retention characteristics, and contributes significantly to separation errors. Because of the variation in the activity produced water deactivated Silica Gel is considered unsuitable for the routine separation of OCPs and PCBs.
The capacity of the optimised clean-up methods investigated is summarised in Table 59.

Ready made Aminopropyl bonded silica columns are convenient, quick and easy to use.
20mls of hexane is the optimum elution solvent for the cartridges. The cartridges have a poor capacity for the removal of coextracted material from sediment extracts. Large scale Aminopropyl bonded silica cartridges are difficult to handle and secondary interactions with the bonded phase give poor recoveries of ε HCH. These cartridges are not suitable for the routine clean-up of environmental extracts.

Table 59 Capacities of Adsorption Chromatography Clean-Ups.

	Maximum Weight (mg) of Coextracted Material Retained on Column		
Clean-up Column	Cod Oil	Mussel Extract	Clyde Sediment Extract
500 mg Aminopropyl Cartridge	28	32	11
500 mg Florisil Cartridge	28	29	29
2 g Acidic, 1 g Basic 7 % Deactivated Alumina Column	21	14	9
6 g 10 % Deactivated Alumina Column	94	80	39
6 g 7.5 % w/w Silver Nitrate 10% Deactivated Alumina Column	103	85	74

Ready made 500mg florisil cartridges are convenient, quick and easy to use. 20mls of 10% MTBE in hexane is the optimum elution solvent for the cartridge. The capacity of the florisil cartridges is similar to the Aminopropyl cartridges for Cod Oil and Mussel extracts. Florisil cartridges have a capacity twice that of the Aminopropyl cartridges for sediment co-extractants.

Glass columns (500mm * 10mm) containing 2 g of Acidic and 1g of Basic 7% water deactivated alumina were optimised. 35 mls of 10% MTBE in hexane was found to be the optimum elution solvent. These columns were time consuming to prepare and had the lowest capacity of all the clean-ups investigated for the removal of coextracted compounds.

Glass columns (500mm * 10mm) containing 6g of 10% water deactivated Neutral alumina were optimised. 100mls of hexane was found to be the optimum elution solvent. The temperature of activation of the alumina was investigated and 500 °C was found to be the optimum temperature. These columns had a high capacity for the removal of co-extractants from cod oil and mussel extracts, their capacity was only slightly greater than 500mg florisil cartridges for sediment extracts. These columns were considerably more time consuming to prepare and use than the florisil cartridges.

Glass columns (500mm * 10mm) containing 6g of 10% water deactivated Neutral alumina impregnated with 7.5 % w/w silver nitrate were optimised. 100mls of hexane was found to be the optimum elution solvent. These columns had a high capacity for the removal of co-extractants from cod oil, mussel and sediment extracts. This was the only clean-up method to remove all the colour from the sediment extracts. These columns were extremely difficult to prepare and use on a routine basis. The silver nitrate alumina had to be used the same day as it was prepared and great care had to be taken to avoid contact with the silver nitrate impregnated alumina.

The limited capacity of the Aminopropyl bonded silica and Acid/Base alumina clean-ups excluded them from consideration as routine clean-up methods. Florisil Cartridges, 6g 10 % water deactivated Neutral alumina and 6 g 10 % water deactivated Neutral alumina impregnated 7.5 % w/w silver nitrate all offered advantages for specific matrices. As indicated in section 3.1 the initial aim was to develop a series of modular clean-up steps which could be mixed and matched as particular sample types required. Outlined below are the clean-up strategies which will be most effective for a variety of matrices.

River waters typically contain only a few mg/L of Total Organic Carbon (TOC) (Dojlid & Best, 1993), this is much less than the adsorptive capacity of a florisil cartridge. As the florisil cartridge is the most convenient, quickest and easiest to use of the suitable methods it is the obvious choice for the clean-up of river water extracts.

Effluents from sewage treatment works typically contain tens of mg/l of TOC. If the majority of organic compounds in the original sample were present in the sample extract it is possible that a florisil column may become overloaded when cleaning -up such an extract. Experience with the test matrices indicated that it was possible to tell by visual inspection of the cartridge when overloading had occurred. Therefore, florisil cartridges were selected as the clean-up method for effluents from STW, if the cartridge became overloaded the clean-up with the florisil cartridge would be repeated. Repeating the florisil clean-up would still be more convenient, quicker and easier than preparing and using a neutral or silver nitrate impregnated alumina column.

Effluents from long sea outfalls which have received only maceration as a treatment typically contain hundreds of mg/L of TOC, in addition they frequently contain high levels of sulphur and Sulphur containing compounds. The coextracted interferences present in extracts from these samples will closely resemble those from sediments. Only silver nitrate impregnated alumina was effective at removing the colour from these extracts. Therefore, silver nitrate impregnated alumina was selected as the most suitable clean-up for these complex extracts. If after this clean-up the extract still appeared coloured a florisil cartridge could be used to remove any residual coextracted compounds.

The main coextracted interference's in biota samples are lipids. Florisil cartridges have a limited capacity for the removal of lipid. Approximately 3 florisil cartridges would have to be used to remove a similar quantity of lipid as one Neutral or silver nitrate impregnated alumina column. The time required to perform 3 florisil cartridge clean-ups and reduce the solvent volume between each clean-up is similar to the time required to prepare and use alumina columns. Each step of extract manipulation and transfer is potentially a point at which analytes could be lost or samples become cross contaminated. To minimise these potential errors an alumina based clean-up was selected as the most appropriate for biota samples. There was only a slight difference in the capacity of neutral and silver nitrate impregnated alumina for the removal of cod oil lipids. As the silver nitrate

impregnated alumina is both costly, difficult and potentially more hazardous to prepare Neutral alumina was selected for the clean-up of biota samples. The most efficient means of removing large amounts of lipid was using multiple alumina columns in parallel, each column loaded with a small portion of the extract (e.g. a portion containing less than 80mg of lipid).

3.5 Optimisation of Destructive Clean-up Techniques

3.5.1 Sulphur Removal

Elemental sulphur is present in most sediments and sewage effluents, sulphur compounds are extremely electron capturing and therefore must be removed from sample extracts prior to GC analysis. Two methods of sulphur removal were investigated: 1) Ion pair reaction with tetrabutyl ammonium sulphate ("Jensens" Reagent) to form thiosulphite (Jensen *et al* 1977), 2) reaction with copper to form copper sulphide.

i) Investigation of Ion pair reaction

Preparation of "Jensens" Reagent: 3.5 g of tert butyl ammonium sulphate was dissolved in 100mls of deionised water. 25 g of sodium sulphite was added and the solution shaken vigorously, then further 5 g portions of sodium sulphite were added to the solution until a precipitate remained. In order to remove impurities in the tert butyl ammonium sulphate the solution is extracted twice with hexane prior to use. The tert butyl ammonium sulphite solution is only stable for 24 hours.

5ml portions of hexane were spiked with the target analytes as per previous tests and 100mg of elemental sulphur, these spiked hexane extracts were shaken vigorously with 3mls of "Jensens" reagent and 1ml of iso propanol. Using a pasteur pipette the upper hexane phase was removed, the remaining aqueous phase was re-extracted with a further 5ml portion of hexane. This hexane phase was removed and combined with the previous hexane extract. The hexane extracts were reduced in volume by TurboVap concentration. The recovery of the target analytes was determined by GC/ECD analysis of the extract. The procedure was repeated 6 times mean recoveries are listed in Table 60.

This method was relatively time consuming to perform, however, the method was extremely efficient with no traces of sulphur detected in the chromatograms and provided quantitative recoveries of the target analytes.

ii) Investigation of Reaction with Copper

Copper powder was cleaned by washing with dilute hydrochloric acid, deionised water and hexane. 1g portions of the cleaned copper powder were added to 5ml portions of hexane which had been spiked with the target analytes and 100mg of elemental sulphur. The extracts plus copper powder were shaken vigorously and allowed to stand for approximately 1 hour. Using a pasteur pipette the hexane extract was removed from the copper powder and reduced in volume by TurboVap concentration. The recovery of the target analytes was determined by GC/ECD analysis of the extract. The procedure was repeated 6 times mean recoveries are listed in Table 60.

The method was quick and easy to perform, providing excellent recoveries of all of the target analytes. However, traces of sulphur were detected in all of the chromatograms.

Table 60 Mean % Recoveries after Sulphur Removal Using "Jensens" Reagent and Active Copper.

Compound	Mean % Recovery using "Jensens" Reagent	Mean % Recovery using active copper
HCBD	89	98
HCB	91	102
γ HCH	89	106
PCB 53	96	97
PCB 52	101	95
Aldrin	98	96
PCB 101	103	99
PP DDE	96	100
Dieldrin	95	99
Endrin	93	99
PP DDT	90	96
PCB 180	101	96
Cis Permethrin	97	103
Trans Permethrin	94	100

3.5.2 Conclusions

Quantitative recoveries were obtained using both these methods, reaction with the ion pairing reagent tert butyl ammonium sulphate provided more complete removal of sulphur. Therefore, reaction with "Jensens" reagent was selected as the method of choice for the removal of sulphur from sewage extracts.

Reaction with copper powder did offer some removal of sulphur, this reaction showed promise as a gross removal step for sediment samples. Active copper powder added to the extraction solvent in the round bottom flask prior to commencing soxhlet extraction of sediments would act as an anti bumping agent and the hot extraction solvent would provide an energetic environment for the reaction between extract sulphur and the copper. This method of treatment was selected for the removal of sulphur from sediment matrixs.

3.6 Optimisation of Extraction Techniques

As outlined in section 2.4.1 the aim of an extraction is to quantitatively remove/extract all of the target analytes from the sample matrix. Methods for determining the extraction efficiency of a particular analytical method are one of the more difficult and ill-defined areas of analytical chemistry. The basic requirement is to determine how much of the analytes have been recovered from the sample matrix by the extraction technique. Determination of recovery requires a knowledge of the amount of analyte present in the sample. Recovery of added target analytes "Spiked " to a sample matrix in an organic solvent immediately prior to extraction does not necessarily give information on the extraction efficiency. To determine extraction efficiency the analytes must be bound to the sample matrix in a manner similar to that which exists in the environment.

This can be to some degree approximated for with aqueous based samples. The target analytes may be added below the surface of the sample in a small (e.g. 1-2ml) volume of water miscible solvent and following mixing the analytes are allowed to come into equilibrium with other organic material by standing for a period of time prior to extraction. The spiked sample must be analysed in its entirety, including all inner surfaces of the container

There are no universally accepted methods for spiking sediment and biota matrices.
A method which may be used with wet sediments, is the addition of the target analytes to the sediments interstitial water. The sediment and pore water are thoroughly mixed and allowed time to equilibrate, the pore water is then removed by decanting or freeze drying. It is assumed that lipophilic compounds are completely associated with the sediment allowing the analysis of the sediment to provide information on the analyte recovery. This method assumes that none of the target analytes are present in the original sediment, the equilibration conditions in the laboratory are equivalent to those found in the environment and the analytes are quantitatively associated with the sediment.
The problems are greater for biological tissue. Organic contaminants can only be fully bound into biota by feeding or exposure studies. The animal distributes the target analytes throughout its body, partitioning and metabolising the analytes in the normal manner. Such feeding or exposure studies do not give information on the quantity of the target analytes bound by the organism and are therefore of limited use in the preparation of recovery test matrices.

The methods described for spiking sediment and biota are extremely time consuming and can rarely be justified in terms of determining the extraction efficiency alone. An estimate of extraction efficiency in sediment and biota matrices may be obtained by selecting the analytical protocols which give the highest concentrations from a homogenous natural matrix. Sequential extractions of the same sample by different analytical protocols may also be used to determine the extraction efficiency. Neither of these techniques directly measures the extraction efficiency but used in

combination they are the most cost effective and practicable means of investigating extraction efficiency.

The analytical strategy for optimising extraction efficiency is matrix dependent. Aqueous matrices (e.g. River waters, and Sewage effluents) and sediment and biota samples are optimised separately. River waters, and sewage effluents are optimised using spiked water samples. Sediment and biota matrices are optimised using natural samples.

3.6.1 Optimisation of Aqueous Extraction Methods.

Section 2.4 discusses the large number of extraction methods that are commonly used in trace organic analysis today. From this review of current analytical extraction practices it is clear that liquid-liquid extraction and solid phase extraction are the two methods most suited for the extraction of a range of contaminants in a multi-residue method . In this section the suitability of these techniques for the extraction of a wide variety of polychlorinated biphenyl's, organochlorine pesticides and synthetic pyrethroids from river waters, effluents from sewage treatment works and long sea outfalls is investigated.

3.6.2 Liquid - Liquid Extraction

DCM and hexane are two of the most commonly used solvents for liquid-liquid extraction and they have been successfully used for extracting a wide range of organic compounds from a variety of aqueous samples. Investigations were carried out to determine which of these solvents would best meet the requirement of providing quantitative recoveries for a wide range of determinands.

3.6.2.1 Comparison of DCM and Hexane as Extraction Solvents.

An extraction recovery standard containing the target analytes outlined in section 3.1 was prepared in acetone. Acetone was selected as the standard solvent because of the target analytes high solubility in it and its high solubility in water .This extraction recovery standard was used for all subsequent aqueous extraction recovery tests. 1ml of the extraction recovery standard prepared as described in section 3.2.4.2 was added to 1L of deionised water in a Pyrex glass sampling bottle. The resulting solutions were shaken thoroughly and allowed to equilibrate for at least two hours. The solutions were extracted twice with either DCM or Hexane, The extracts were dried by passing through anhydrous sodium sulphate and reduced in volume by TurboVap concentration. The % recovery of the target analytes was determined by GC/ECD analysis of the extract as described in section 3.2.

Experimental Procedure

1. Eight 1 litre Pyrex glass bottles fitted with PTFE lined caps were washed thoroughly with warm water and Decon. The bottles were rinsed thoroughly with deionised water, Acetone and either Hexane or DCM. The clean bottles were dried in an oven at 35- 40 C before use.

2. Approximately 1 litre of MiliQ organic free water was added to each bottle, using a glass syringe 1 ml of the extraction recovery standard was added to six of the bottles. All the bottles were sealed shaken thoroughly and allowed to equilibrate for at least two hours.

3. The bottles were split into two Batches (A and B) of 3 spiked and 1 blank samples. 50mls of hexane was added to Batch A and 50 mls of DCM to Batch B. All the samples were shaken thoroughly by hand for at least two minutes.

4. The samples plus solvent were quantitatively transferred to clean 1 L separatory funnels, and the layers allowed to separate for approx. 1min.

5. For Batch A samples the lower aqueous layer was transferred back into the original sample bottle, the upper hexane layer was transferred through a filter paper containing approx. 2 g of anhydrous sodium sulphate into a TurboVap tube.
   For Batch B the lower DCM layer was transferred through a filter paper containing approx. 2 g of anhydrous sodium sulphate into a TurboVap tube, the upper aqueous layer was then transferred back into the original sample bottle.

6. A second aliquot of 50 ml of Hexane was added to Batch A sample and 50 ml of DCM to batch B samples. All the samples were shaken by hand for at least two minutes.

7. Steps 4 and 5 were repeated.

8. The volume of the extracts were reduced to approx. 1 mls by TurboVap concentration. Using a glass syringe 0.5mls of iso octane was added to the TurboVap tube and the extract reduced in volume to 0.5mls. The extracts were quantitatively transferred to a GC vial, made up to 1 ml with iso-octane and internal standards added. The extracts were analysed by GC/ECD as described in section 3.2.

The mean % recovery of each analyte for the 3 blank corrected determinations are shown in Table 61.

Table 61. % Recovery of PCBs and OCPs from One Litre of Deionised water extracted with either DCM or Hexane.

	Mean % Recovery	
Compound	Two 50 ml Hexane Extractions	Two 50ml DCM Extractions
HCBD	89.1	84.3
HCB	73.5	72.9
G HCH	91.3	88.6
E HCH	89.8	84.3
PCB 53	78.3	79.1
PCB 52	210	240
Aldrin	79.5	77.6
PCB 101	75.3	73.4
PP DDE	76.1	77.2
Dieldrin	75.8	74.9
Endrin	86.7	88.5
PP DDT	83.0	83.9
PCB 180	83.8	88.6
Cis Permethrin	92.1	94.3
Trans Permethrin	92.8	90.7

The results show quantitative recovery of all of the test analytes by both DCM and Hexane extraction. No significant difference is observed in the extraction efficiencies of the two solvents. Both sets of extracts gave complex chromatograms with numerous unidentified peaks. A large interfering peak was detected in the immediate area around PCB 52. The blank was unable to correct for this interference and a grossly elevated recovery is observed for this compound. Subsequent tests demonstrated that the Whatman No 1 filter papers contained significant amounts of electron capturing materials. These materials were washed into the extract during the drying step and this contamination from these materials resulted in grossly elevated recoveries for PCB 52. In all subsequent tests these interference's were removed by soaking the filter papers in solvent prior to use.

The two solvents showed no significant difference in extraction efficiency and therefore other criteria were used to select the solvent for routine use. DCM is a suspected carcinogen, and is 10 times more expensive than hexane. DCM carries the additional risk that possible residues from the evaporation step could contaminate the ECD detector. Because of these factors DCM was rejected and Hexane was chosen as the solvent for routine extraction of aqueous samples.

3.6.2.2 Optimisation of sample size on Extraction Efficiency

During the previous experiment it was noted that there was poor mixing of the organic and aqueous phases. Tests were undertaken to determine whether the extraction solvent / sample size / bottle size ratios affected extraction efficiency.

1ml of the extraction recovery standard was added to 500ml of deionised water in a 1L Pyrex glass sample bottle and extracted twice with 50 mls of hexane. The extracts were dried by passing through anhydrous sodium sulphate and reduced in volume by TurboVap concentration. The % recovery of the target analytes was determined by GC/ECD analysis of the extract as described in section 3.2.

The experimental procedure described for Batch A in section 3.6.2.1 was carried out.

The mean % recovery of the target analytes from the 3 determinations are shown with the previous hexane extraction results.

Table 62 % Recovery of PCBs and OCPs from 500ml and 1L of deionised water.

	Mean % Recovery	
Compound	Two 50 ml Hexane Extractions on 1 litre Sample	Two 50ml Hexane Extractions on 500ml Sample
HCBD	89.1	73.2
HCB	73.5	65.9
γ HCH	91.3	75.0
ε HCH	89.8	75.0
PCB 53	78.3	63.6
PCB 52	210	76.3
Aldrin	79.5	71.6
PCB 101	75.3	70.4
PP DDE	76.1	72.3
Dieldrin	75.8	74.9
Endrin	86.7	88.5
PP DDT	83.0	83.9
PCB 180	83.8	78.0
Cis Permethrin	92.1	87.9
Trans Permethrin	92.8	91.9

Acceptable recoveries are achieved for all of the target analytes. Surprisingly lower recoveries are observed for the increased ratio of solvent to sample extractions. This result was unexpected. A possible explanation is that the results from the first tests are high. These results may have been over quantified by the effect of the electron capturing compounds co-extracted from the filter papers in the first test. The differences are greater for the lighter compounds. These compounds occur in the area of the chromatogram where the interferences from the filter paper were greatest. It is likely that the higher recoveries found in the first batch are due to the presence of interfering compounds from the filter paper. When consideration is given to the contamination problems the differences

between the extraction efficiencies are small indicating that adequate mixing of the two phases is occurring with the 1L sample size.

3.6.2.3 Investigation of Effect of Salt on Extraction Efficiency

The recoveries obtained using a double extraction of hexane are acceptable but could be improved. Lowering the water solubility of the target analytes by the addition of salt to increase the efficiency extraction is a common technique in organic analysis. Test were carried out to determine whether this technique would improve PCB and OCP extraction efficiency.

1ml of the extraction recovery standard was added to 1 L of deionised water in a 1L Pyrex glass sample bottle and allowed to equilibrate for at least two hours. 28g of sodium chloride was added to the sample which was then extracted twice with 50 mls of hexane. The extracts were dried by passing through anhydrous sodium sulphate and reduced in volume by TurboVap concentration. The % recovery of the target analytes was determined by GC/ECD analysis of the extracts as described in section 3.2.

The experimental procedure was as described in section 3.6.2.1 for batch A with the addition of 28 g of sodium chloride to the sample at step 3 immediately before the addition of the first 50mls of hexane.

The mean % recoveries of the target analytes of a salt extraction compared with the standard extraction carried in section 3.6.2.1 are shown in Table 63.

Table 63% Recovery of PCBs and OCPs from salt fortified deionised water.

	Mean % Recovery	
Compound	Two 50 ml Hexane Extractions on 1 litre Sample	Two 50ml Hexane Extractions on 1 Litre NaCL fortified Sample
HCBD	89.1	91.2
HCB	73.5	73.2
γ HCH	91.3	87.5
ε HCH	89.8	90.1
PCB 53	78.3	89.7
PCB 52	210	93.2
Aldrin	79.5	86.2
PCB 101	75.3	83.7
PP DDE	76.3	79.1
Dieldrin	75.8	95.1
Endrin	86.7	86.3
PP DDT	83.0	82.7
PCB 180	83.8	88.7
Cis Permethrin	92.1	89.25
Trans Permethrin	92.8	90.37
Mean Recovery	82.81*	87.09

* Excluding PCB 52

Acceptable recoveries are obtained for the target analytes and improvement is observed by the addition of salt. The improvement is small, but the possibility of sample contamination by the addition of the salt makes this technique unacceptable.

3.6.2.4 Conclusion

The optimum liquid liquid extraction conditions for a 1L water sample in a 1L sample bottle were determined as a double 50ml extraction with hexane carried out as described in 3.6.2.1.

3.6.3 Solid Phase Extraction

There is increasing interest in the use of solid-phase adsorbents for the removal and concentration of trace organic pollutants from water samples. As discussed in the method review polystyrene/divinylbenzene resins, activated carbon, and polyurethane foam have all been used as absorbents with varying degrees of success. Bonded silicas have recently emerged as the most promising of adsorbents for the routine trace extraction of a wide variety of organic molecules from environmental water samples.

Bonded -phase silicas have several advantages over other sorbents. Silica gel provides a rigid substrate that forms a bed that will not distort when pressure is applied for rapid fluid flow. In comparison to resins bonded silica does not require solvent or buffer conditioning, and silica particles do not significantly change in size with change in solvent composition. By varying the chemical moiety bonded to the silica surface a degree of specificity to the compound of interest may be obtained. SPE is an emerging technique with more than 30 suppliers offering bonded silica phases ranging from conventional HPLC phases, such as C18, C8, cyano and amino, to reactive particles that users can derivatize with a ligand of their choice (Markell, *et al.*, 1991)

The bonded silica most widely used for environmental applications is C18. C18 is a non polar bonded sorbent which uses classical reverse phase chromatography interactions, i.e. the sorbent (C18 bonded silica) is less polar than the mobile phase or sample solution. Non polar analytes are partitioned from the sample solution by non-polar interactions such as van der Waals or dispersion forces. The analytes can then be eluted from the column by a solvent having a non-polar character such as hexane or DCM which is capable of disrupting the nonpolar interactions.

A disadvantage of this adsorption process is that it is very non selective, since most organic molecules have the potential for non polar interactions. Exceptions include inorganic ions and compounds (carbohydrates for example) whose structure contain sufficient polar or ionic groups to mask the carbon structure of the molecule.

The suitability of C18 solid phase extraction cartridges for the extraction of a wide variety of polychlorinated biphenyl's, organochlorine pesticides and synthetic pyrethroids from river waters, sewage effluents and trade effluents was tested.
A solid phase extraction procedure typically involves five steps;

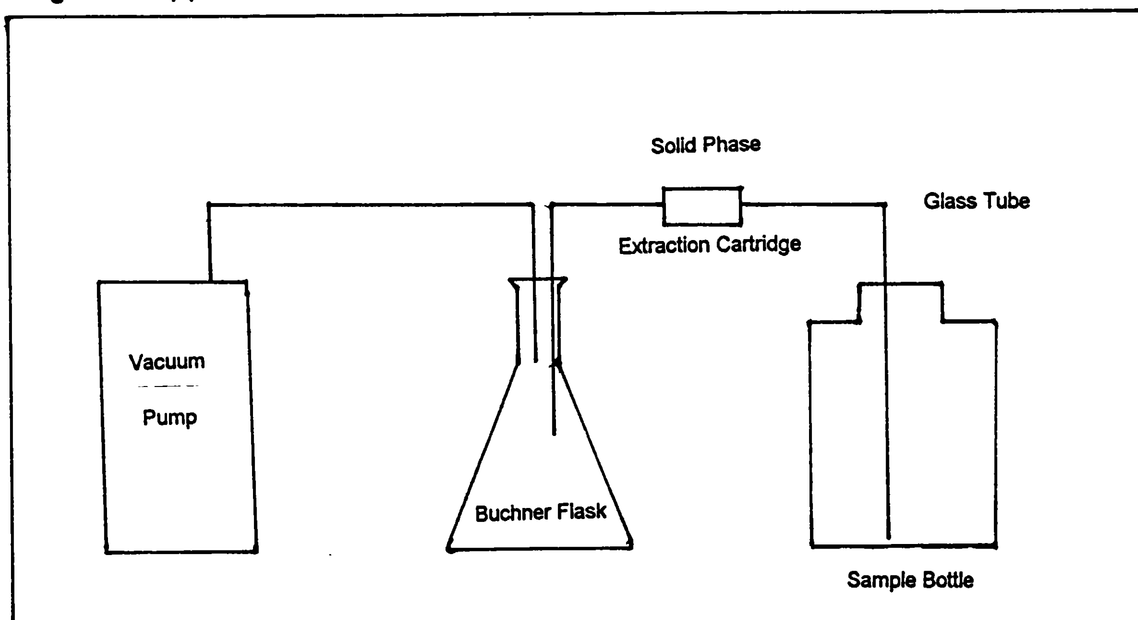
1. Sample pre-treatment.
2. Column conditioning.
3. Column pre-equilibrium.
4. Sample application.
5. Analyte elution.

Each of these steps were examined in detail.

3.6.3.1 Preliminary Investigation of C18 Bonded Silica Extraction Cartridges.

Literature from manufacturers of bonded sorbents provided a useful starting point for method development. Bakerbond and Varian both provide application notes outlining procedures for the extraction of organochlorine compounds from potable waters. These procedures use 100mg C18 columns with hexane as the solvent for analyte elution. 1ml of the extraction analyte recovery standard prepared as described in 3.2.4.2 was added to 1L of deionised water in a Pyrex glass sampling bottle. The resulting solution was shaken thoroughly and allowed to equilibrate for at least two hours. A slightly modified version of the Varian procedure utilising a vacuum pump was used for the extraction and analyte recovery. The hexane extracts were dried by passing through anhydrous sodium sulphate and reduced in volume by TurboVap concentration. The % recovery of the target analytes was determined by GC/ECD analysis of the extract as described in section 3.2.
The following apparatus was used to pass the sample through the cartridge.

Figure 9 Apparatus for Solid Phase Extraction of Aqueous Samples



Experimental Procedure

1. Four 1 litre Pyrex glass bottles fitted with PTFE lined caps were washed thoroughly with warm water and Decon. The bottles were rinsed thoroughly with deionised water, Acetone and either Hexane or DCM. The clean bottles were dried in an oven at 35- 40 °C before use.

2. Approximately 1 litre of MiliQ organic free water was added to each bottle, using a glass syringe 1 ml of the extraction recovery standard was added to 3 of the bottles. 1ml of Acetone was added to the 4th which was a reagent blank. All the bottles were sealed shaken thoroughly and allowed to equilibrate for at least two hours.

3. 50mls of Methanol was added to all the samples. The bottles were sealed and shaken thoroughly.

4. Using a large glass syringe the cartridge was cleaned by passing approx. 10mls of Hexane at a flow rate of 5-10mls a minute trough the cartridge.

5. The cartridge was conditioned by passing two approx. 10ml portions of methanol through the cartridge at a flow rate of 5-10ml a minute.

6. The cartridge was equilibrated by passing approx. 10ml of deionised water through the cartridge at 5-10mls a minute. This also removes any excess methanol.

7. The cartridge was now loaded into the extraction apparatus shown in Figure 9, the glass tube was placed into the sample bottle and the vacuum pump switched on. The valve was used to adjust the vacuum pressure to allow the loading of the sample onto the column at a rate of approximately 33ml/min.

8. Once all of the sample was loaded onto the column, air was sucked through the cartridge for a further 10min to dry the cartridge.

9. The cartridge was removed from the extraction apparatus. Using a large glass syringe two 5 ml portions of hexane were passed through the cartridge at 5ml/min. The two Hexane elutes were collected in a small TurboVap tube.

10. The volume of the extracts were reduced to approx. 1 mls by TurboVap concentration. Using a glass syringe 0.5mls of iso octane was added to the TurboVap tube and the extract reduced in volume to 0.5mls. The extracts were quantitatively transferred to a GC vial and made up to 1 ml with iso-octane. The extracts were analysed by GC/ECD as described in section 3.2

The % recovery of each analyte was calculated. The mean result of the 3 determinations corrected for the blank is shown in Table 64.

Table 64. % Recovery of PCBs and OCPs from One Litre of Deionised water using C18 SPE with Hexane as Elution Solvent.

Compound	% recovery
α HCH	67
HCB	57
γ HCH	62
ε HCH	67
PCB 53	55
PCB 52	61
Aldrin	53
PCB 101	58
Dieldrin	64
Endrin	60
PP DDT	59
PCB 180	51
Cis Permethrin	63
Trans Permethrin	66

Manufacturer's literature suggests that recoveries of 85 % could be achieved using C18 SPE cartridges. The recoveries obtained in these tests are much lower. These initial test recoveries were disappointing. The tests confirm the extraction of PCBs and OCPs from water using solid phase extraction and suggested that the technique was worthy of further investigation.
Problems had been experienced in passing the sample through the cartridge. The initial flow rate of 33mls a minute deteriorated as sample was passed through the cartridge. The vacuum had to be progressively increased and after approx. 600mls of sample had passed through the cartridge full vacuum was being applied. As more sample was passed through the cartridge the sample loading rate reduced to approximately 15 mls/min.

The vacuum pump used for the preliminary investigation was an old pump of unknown capacity. The preliminary test demonstrated that this pump was not suitable for the processing of large numbers of samples. To eliminate this pump as a possible cause of the poor recoveries the test was repeated using a modern Edward's rotary vacuum pump.

In order to maximise the amount of information from this test the water samples were spiked at double the previous spiking level. This allowed hexane liquid-liquid extraction of the residue of the spike left in the sample after it had passed through the extraction cartridge. Assuming similar recoveries to those obtained in the previous tests the liquid liquid extraction would provide information on the % of analytes remaining in solution following solid phase extraction.

Equipment is as described previously with the substitution of a Edward's vacuum pump. The experimental procedure is similar to the procedure described previously. On completion of step 8 the aqueous sample collected in the Buchner funnel was transferred to a separatory funnel, the buchner funnel was rinsed with 50 mls of hexane. This and an additional 50ml aliquot of hexane were used to perform a liquid-liquid extraction on the aqueous sample using the procedure optimised in section 3.6.2.4.

The mean results of the 3 determinations corrected for the blank are shown in Table 65. Recoveries of less than 10 % were not quantified.

Table 65. % Recovery of PCBs and OCPs from One Litre of Deionised Water using C18 SPE with Hexane as Elution Solvent.

Compound	% of PCBs and OCPs recovered from cartridge	% of PCBs and OCPs recovered from SP "extracted" water.
α HCH	64	13
HCB	59	11
γ HCH	57	17
ε HCH	70	<10
PCB 53	56	<10
PCB 52	58	<10
Aldrin	53	17
PCB 101	54	ND
Dieldrin	60	17
Endrin	66	14
PP DDT	52	18
PCB 180	53	<10
Cis Permethrin	66	<10
Trans Permethrin	68	<10

Where ND is not detected.

The new pump improved the flow of water through the cartridge. Full vacuum was required in the later stages to maintain a flow of 33ml/min. The recoveries obtained from this experiment are similar to those obtained in the preliminary test. This indicates that the poor pump performance and hence flow rate through the cartridge does not have a major effect on analyte recovery. The PCBs and OCPs were not detected in significant quantities in the aqueous portion of the sample after it had passed through the extraction cartridge. This suggests that a significant portion of the PCBs and OCPs were either not eluted by hexane and retained on the extraction cartridge or were bound to the glass or tubing in the sampling bottle and extraction apparatus.

3.6.3.2 Optimisation of analyte elution from C18 extraction Cartridges.

Other workers (Van Horne, 1985) have suggested that a possible mechanism for the poor elution of non-polar analytes from non-polar sorbents is insufficient drying of the sorbent. During the extraction step small amounts of residual water are trapped in and around the C18 phase. The trapped water molecules repel the non-polar (hexane) elution solvent preventing it reaching the analytes thus blocking recovery. To overcome this problem a two stage mixed polarity elution may be used. The C18 sorbent is initially eluted with a medium polarity water miscible solvent such as ethyl acetate, residual water and the most polar analytes will be removed by this solvent stage. The remaining very non polar analytes are now removed by a nonpolar solvent such as hexane.

The experimental procedure is similar to the procedure described previously. At step 9 however, the extraction cartridge is eluted with 5 mls of Ethyl Acetate followed by 5mls of hexane. The eluates are combined, dried and reduced in volume before GC/ECD determination of the % recovery as described in 3.2.

The mean results of the 3 determinations corrected for the blank are shown in Table 66.

Table 66 % Recovery of PCBs and OCPs from One Litre of Deionised Water using C18 SPE with Ethyl Acetate and Hexane as Elution Solvent.

Compound	% of PCBs and OCPs recovered from cartridge
α HCH	68
HCB	66
γ HCH	61
ε HCH	84
PCB 53	81
PCB 52	80
Aldrin	83
THE	79
PCB 101	76
Dieldrin	85
Endrin	84
PP DDT	81
PCB 180	74
Cis Perm	88
Trans Perm	89
DCBP	77

These results show a significant improvement with the addition of the ethyl acetate elution step. This confirms that the earlier poor recoveries were due to analyte retention on the cartridge. A slight gradient in the recoveries is observed, with the light compounds such as α HCH and HCB showing slightly lower recoveries than the heavier compounds cis and trans permethrin. The recoveries obtained by this method are quantitative but not as high as those obtained by conventional hexane liquid-liquid extraction.

3.6.3.3 Optimisation of Analyte Recovery from Spiked River Water.

For ease of operation the preliminary tests were on spiked deionised water. This is an unrealistic test since deionised water is a pure matrix containing low levels of dissolved organic compounds and no suspended material. An advantage of deionised water is that the extracts can be analysed directly without clean-up. This reduces the possible sources of error such as retention on clean-up columns. Dissolved organic compounds and suspended material may reduce recoveries to less than previous test have indicated. Thus tests on spiked river water allow a more realistic appraisal of the extraction techniques.

Four 2.5L Winchesters of river water were collected from the White Cart Water at the Eaglesham road bridge. Biochemical oxygen demand and suspended solids analysis were used to characterise the water. Portions of the river water were spiked with the test analytes and the concentrations in the river water determined using the analytical protocol optimised in section 3.6.2.

1. Seven 1 litre Pyrex glass bottles fitted with PTFE lined caps were washed thoroughly with warm water and Decon. The bottles were rinsed thoroughly with deionised water, acetone and either hexane or DCM. The clean bottles were dried in an oven at 35- 40°C before use.

2. The 4 Winchesters of River water were shaken thoroughly by hand. Approximately 250ml portions of river water were decanted into each sample bottle from each Winchester. The sample bottles were sealed and shaken thoroughly, one sample bottle was sent for Biochemical oxygen demand and suspended solids analysis. The remaining bottles were split into two batches of three (Batch A and Batch B). Batch A was spiked with 1 ml of the extraction recovery standard using a glass syringe, Batch B was spiked with 1ml of Acetone using a glass syringe. Both batches were sealed shaken thoroughly and allowed to equilibrate for at least two hours.

3. 50mls of Methanol was added to both batches of samples. The bottles were sealed and shaken thoroughly.

4. Using a large glass syringe the cartridge is cleaned by passing approx. 10mls of Hexane at a flow rate of 5-10mls a minute through the cartridge.

5. The cartridge is condition or solvated by passing two approx. 10ml portions of methanol through the cartridge at a flow rate of 5-10ml a minute.

6. The cartridge is equilibrated by passing approx. 10ml of deionised water through the cartridge at 5-10mls a minute. This also removes any excess methanol.

7. The cartridge was now loaded into the extraction apparatus described in section 3.6.3.1, the glass tube was placed into the sample bottle and the vacuum pump switched on. The valve was

used to adjust the vacuum pressure to allow the loading of the sample onto the column at a rate of approximately 33ml/min.

8. Once all of the sample was loaded onto the column, air was sucked through the cartridge for a further 10min to dry the cartridge.

9. The cartridge was removed from the extraction apparatus. Using a large glass syringe a 5 ml portion of ethyl acetate was passed through the cartridge at 5ml/min followed by a 5ml portion of hexane. Both eluates were collected in the same small TurboVap tube.

10. The volume of the extracts were reduced to approx. 1 mls by TurboVap concentration. Using a glass syringe 0.5mls of iso octane was added to the TurboVap tube and the extract reduced in volume to 0.5mls. The extracts were quantitatively transferred to a GC vial and made up to 1 ml with iso-octane. The extracts were analysed by GC/ECD as described in section 3.2. Steps 4 to 10 were repeated for each sample in both batches.

The results for suspended solids and BOD analysis of the river water are shown below.

BOD	2.6mg/L
Suspended Solids	17mg/L

Extreme difficulties were encountered when trying to pass 1 litre of river water through the solid phase extraction cartridge. The rate at which the sample passed through the cartridge slowed dramatically as more sample was loaded onto the cartridge. After approximately 100 mls of sample had been loaded onto the cartridge the vacuum valve was fully open, the pump operating at maximum power, and the sample loading rate continued to slow. After approx. 3 hours only 150mls of sample had been loaded onto the column and the sample loading appeared to have come to a complete halt. At this stage the sample loading was abandoned. Unfortunately the cartridge could not be eluted with solvents.

It was clear from visual inspection of the cartridge that as sample passed through the cartridge suspended material was trapped on the frit at the top of the cartridge and between the particles in the cartridge. Over a relatively short period of time the frit and cartridge had become completely clogged preventing the flow of sample through the column.

Riverine samples contain significant loadings of particulate material and therefore this clogging of the solid phase column with this particulate presents a severe limitation on the use of these columns with "real" samples.

Two possible solutions to this problem are 1) a more powerful vacuum pump or 2) a series of pre-filters prior to the extraction cartridge. The vacuum pump in use was the largest available to this study and therefore option two was investigated.

A 1μm glass fibre filter paper and loosely packed glass fibre wool were packed on top of the cartridge.
This design traps the large particles on the packed glass wool and intermediate sized particle on the glass fibre filter. Only very fine particles pass through on to the cartridge. A second extraction was attempted using the pre-filtered cartridge. These steps improved the rate of loading but after approximately 200mls the loading rate reduced dramatically. The cartridge became completely blocked after approx. 300mls of sample had been loaded onto the cartridge. Elution of the cartridge with solvent was again not possible.

Conclusion
In their present form using a vacuum manifold system the cartridges were not suitable for the extraction of pesticides from river waters. The cartridges become clogged with suspended material when large amounts of river waters were passed through them. The addition of pre-filters to remove suspended material prior to the cartridge gave only a minimal improvement.

3.6.3.4 Optimisation of Empore Extraction Disks
Empore extraction disks consist of chemically bonded silica particles enmeshed in an inert PTFE matrix to create a mechanically stable sorbent disk. Each disk comprises of chemically modified 8 micron silica tightly bound in a densely woven 'web' of micro-PTFE fibrils. The silica particles are individually suspended such that the surface of each particle is free to interact with the solvent and sample during separation. Since the particles are suspended in the PTFE matrix, no binders are necessary to hold them in place. The mechanism and methods of analyte adsorption are the same as for the extraction cartridges, but, the increased surface area of the disk allows processing of samples with suspended solids.

The disks are manufactured in 25mm and 47 mm diameter sizes , the 47mm size was chosen for investigation to give the greatest surface area allowing the highest loading of sample onto the disk.

Previous work on the extraction cartridges had shown that ethyl acetate followed by hexane were the best eluates for the c18 phase and therefore a similar elution method was used for the disk extraction trials. Initial tests were carried out using the spiked river water from section 3.6.3.3. Two methods were investigated, A) extraction using only an Empore extraction disk and B) extraction with an Empore extraction disk with a 1um glass fibre prefilter.

Reagents were as described in section 3.6.3.3 with the addition of Empore C18 47mm diameter extraction disks and membrane filtration apparatus. The experimental protocol was similar to those described in sections 3.6.3.2 and 3.6.3.3

This test was more readily completed with approximately 600mls of sample passed through the disk before it became blocked. The full one litre of river water was passed through the disk using the

Empore disk and glass fibre prefilter. Both of these disks were successfully eluted with ethyl acetate and hexane, the % recoveries are shown below. The recovery for the disk without pre-filter has been corrected to allow for the reduced volume of sample which passed through the disk. The % recovery on the target analytes is listed in Table 67.

These results indicate that the Empore disk is suitable for extracting the target analytes from water samples. The results are similar to those obtained for the extraction of the target analytes from deionised water using the cartridge system. This indicates that the suspended solid loading and naturally occurring dissolved organic compounds have not reduced the recoveries of the target analytes. The suspended loading in this experiment was 17mg/L and even with the glass fibre prefilter it was difficult to pass one litre of river water through the disk. This suggests that this system would not be suitable for processing river waters with high suspended solid loadings or effluents with high suspended solids

Table 67 % Recovery of Analytes from Empore Extraction Disk.

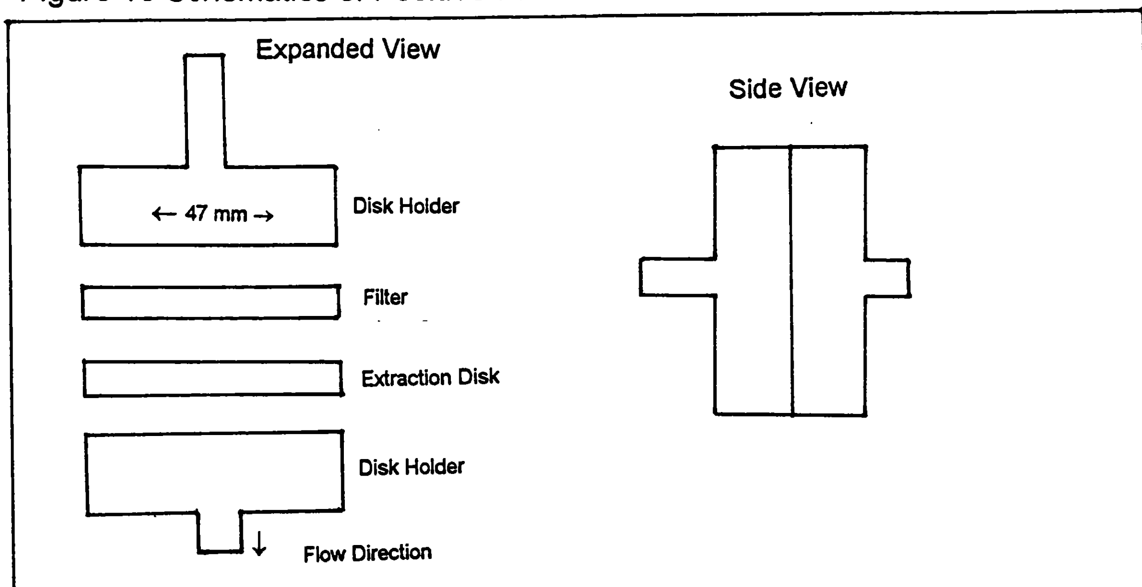
Compound	% Recovery of PCBs and OCPs using Empore extraction disk.	% Recovery of PCBs and OCPs using Empore extraction disk fitted with glass fibre prefilter.
α HCH	62	67
HCB	67	69
γ HCH	50	51
ε HCH	74	88
PCB 53	71	77
PCB 52	69	80
Aldrin	66	81
PCB 101	71	76
Dieldrin	76	82
Endrin	75	81
PP DDT	82	89
PCB 180	70	69
Cis Permethrin	90	91
Trans Permethrin	88	90

3.6.3.5 Positive Pressure Disk Extractions.

In the previous test the maximum vacuum that could be applied was one atmosphere. A positive pressure system allows pressures greater than 1 atmosphere to be applied to force the sample through the disc.

The use of a variety of commercial filter/membrane holders was investigated, however, these were all constructed of materials which would either be attacked by the eluting solvents (ethyl acetate/ hexane) or would strongly adsorb the analytes from water. A disk holder of PTFE was constructed by Cowie scientific, a specialist producer of PTFE scientific apparatus. Figure 10 shows schematics of the disk holder.

Figure 10 Schematics of Positive Pressure Disk Holder.



The aim of these tests was to determine whether positive pressure could be applied to push water samples through an extraction disk. An experimental design similar to that used in section 3.6.3.5 was used for this series of tests. Two units of positive pressure apparatus were constructed, one for handling large volumes i.e. one litre and another for handling smaller volumes of up to 50mls.

1. The river water was collected, spiked and pre-treated with methanol as described previously.

2. An extraction disk and glass fibre prefilter were loaded into the PTFE disk holder as shown in figure 10. Using the small volume positive pressure apparatus the disk was cleaned by pushing 10mls of ethyl acetate, followed by 10mls of hexane through the disk. The rate at which the solvents were pushed through the disk was controlled by altering the compressed air pressure.

3. The disk was conditioned/solvated by passing two approx. 10ml portions of methanol through the disk in a similar manner.

4. The disk holder was transferred to a large volume positive pressure holder and the sample was passed through the disk, the compressed air pressure was gradually increased as more sample was passed through the disk in order to maintain a constant flow rate. Once all the sample had passed trough the disk air was allowed to pass through the disk for a further 10min to dry the disk.

5. The disk holder was transferred to a small volume positive pressure apparatus and the disk eluted with 10mls of ethyl acetate followed by 10mls of hexane. The eluates were combined and dried by passing through anhydrous sodium sulphate.

8. The volume of the combined extracts was reduced to approx. 1 mls by TurboVap concentration. Using a glass syringe 0.5mls of iso octane was added to the TurboVap tube and the extract

8. The volume of the combined extracts was reduced to approx. 1 mls by TurboVap concentration. Using a glass syringe 0.5mls of iso octane was added to the TurboVap tube and the extract reduced in volume to 0.5mls. The extracts were quantitatively transferred to a GC vial and made up to 1 ml with iso-octane. The extracts were analysed by GC/ECD as described in section 3.2.

This method of extracting samples was very successful. One litre of river water was comfortably passed through the disk in approximately 45 mins. The % recovery of the target analytes from river water using this method is given in Table 68.

Table 68 %Recovery of Analytes From River Water using Positive Pressure Empore Disk

Compound	% Recovery of PCBs and OCPs using Empore extraction disk with positive pressure.
α HCH	66
HCB	65
γ HCH	56
ε HCH	72
PCB 53	75
PCB 52	68
Aldrin	67
PCB 101	70
Dieldrin	79
Endrin	781
PP DDT	80
PCB 180	73
Cis Permethrin	94
Trans Permethrin	88

Further tests were carried out with this system. One litre of river and effluent water samples with suspended solids loadings of up to 80ppm were successfully passed through this apparatus in under an hour.

A wide variety of samples were processed using this system, however, it was concluded that this technique was not as suitable for routine use as traditional liquid liquid extraction. The main disadvantages of the empore discs were i) it relied on crudely prepared home made apparatus ii) only one sample could be processed at a time, iii) a high amount of operator intervention was required and iv) pressurising a glass container had safety implications. Solid phase extraction is a technique which shows potential for multi-residue environmental analysis but with the available equipment the method tested was unsuitable for routine use.

3.6.3.6 Automated Solid Phase Extraction

During the research period of this project (1990-1995) solid phase extraction became a widely used technique in applications such as pharmaceutical and clinical analysis where only a small amount of sample was required to be passed through the cartridge. This wider use encouraged manufacturer's to develop new products which would encourage the development of the use of solid phase extraction in different fields of analysis. One such product which became available towards the end of the research period of this project was the Zymark Autotrace SPE workstation. This is a computer controlled automated workstation allowing the simultaneous processing of six samples using positive displacement pumps and automated solvent dispensing. The instrument may be used with solid phase disks or solid phase cartridges. The following section outlines the optimisation of this instrument for the extraction of organochlorine pesticides, polychlorinated biphenyls, and synthetic pyrethroids from aqueous samples.

Application notes from Zymark (Zymark Application Highlight HA4 12/92 and HA3 12/92) indicated that this system had already been successfully applied to the extraction of a wide range of organochlorine pesticides from reagent, raw, and treated water. The system utilises analytical methodologies similar to those previously tested. These are based on a C18 extraction cartridge with Iso- Propyl Alcohol (IPA) activation and analyte elution with ethyl acetate and dichloromethane. The application note indicated that high recoveries were obtained for a variety of organochlorine pesticides. The manufacturers recommendations were rejected for routine work because of health and safety considerations and possible electron capture detector poisoning from residual DCM. Previous method development work had also indicated that many of the compounds of interest to this study were so strongly bound to the C18 phase that elution was difficult. Because of these considerations method development using the automated system were undertaken using Isolute C 8 1g cartridges with a 6ml reservoir volume.

3.6.3.7 Familiarisation with Automated Solid Phase Extraction using the Autotrace System

Initial tests were undertaken to determine if processing real samples with a particulate loading was possible and to gain experience with the operation of the Autotrace system.

Using methods similar to those described previously 3 duplicate 1 L samples (2 of river waters and 1 a crude sewage effluent) described in Table 69, 5 1 L deionised water "samples" spiked with a selection of organochlorine compounds and a 1 L deionised water blank, were pre-treated by the addition of 50mls of iso-propyl alcohol. The samples and blank were mixed thoroughly and processed on the Autotrace using the program listed in Table 70.

Elution was by the previously proven solvent mixture of hexane and ethyl acetate.
The extracts of the spiked deionised water samples, and blank were dried by passing through anhydrous Sodium Sulphate and analysed by GC/ECD and the mean % recovery of each of the target analytes calculated. The results are presented in Table 71.

Table 69. Samples processed using the AUTOTRACE Extraction system.

Sample Site	Suspended Solids mg/L	BOD mg/L
White Cart B769 Road Bridge	13	3.1
Calder Water A 726 Road Bridge	64	5.8
Effluent from Irvine Valley Sewer	127	83

Processing samples with the Autotrace system was much simpler and faster than any of the systems tested previously.
All of the deionised, and river water samples passed through the cartridges. The sample processing rate did not appreciably slow when processing these samples. All of these cartridges were successfully eluted with solvent. Approximately 800mls of the Irvine Valley Sewer sample passed through the cartridge. The processing rate of this sample began to slow after approx. 700mls of sample had been processed. The back pressure regulator system which protects the pumps of the Autotrace system prevented the processing of more than 800 mls of this sample. The cartridge was not eluted with solvent by the Autotrace system, however, the cartridge was eluted manually using a 25ml glass syringe.

Typical river waters and treated sewage effluents in non spate conditions will contain less than 30 mg/L suspended solids and less than 20 mg/L BOD. These tests demonstrate that the Autotrace system is capable of routinely processing the majority of these sample types.

No interfering peaks were observed in the blank. The mean recovery and % RSD of the 5 replicate samples are listed in Table 71.

Table 70 Autotrace Program.

AUTOTRACE EXTRACTION PROCEDURE
Estimated Time For samples 78.9 minutes

Step 1 : Process 6 samples using the following procedure:
Step 2 : Wash syringe with 10ml ETHYL ACETATE
Step 3 : Wash syringe with 10ml IPA
Step 4 : Condition column with 10ml IPA into solvent WASTE
Step 5 : Condition column with 10ml ETHYL ACETATE into solvent WASTE
Step 6 : Condition column with 10ml IPA into solvent WASTE
Step 7 : Condition column with 10ml DEINOISED H2O into solvent AQUEOUS WASTE
Step 8 : Load 1100ml of sample onto column
Step 9 : Rinse column with 10 ml of DEIONISED H2O into AQUEOUS WASTE
Step 10 : Dry column with gas for 2min
Step 11 : Wash syringe with 5ml ETHYL ACETATE
Step 12 : Wash syringe with 5ml ETHYL ACETATE
Step 13 : Soak and Collect 3 ml fraction using ETHYL ACETATE
Step 14 : Collect 3 ml fraction into sample tube using ETHYL ACETATE
Step 15 : Soak and Collect 3 ml fraction using HEXANE
Step 16 : Collect 3 ml fraction into sample tube using HEXANE
Step 17 : END

SETUP PARAMETERS

FLOW RATES

Cond Flow : 40 ml/min
Load Flow : 30 ml/min
Rinse Flow 40 ml/min
Elute Flow : 20 ml/min
Cond Air Push : 40 ml/min
Rinse Air Push :40 ml/min
Elute Air Push :20ml/min

SPE PARAMETERS

Push Delay : 5 sec
Air Factor : 1
Autowash Vol : 0.00 ml

WORKSTATION PARAMETERS
Max. Elution Vol: 20.0 ml
Exhaust Fan on: N
Beeper on: Y

Table 71 % Recoveries of Analytes using the AUTOTRACE Extraction System

Compound	Mean Recovery %	% R.S.D
HCBD	14.0	17.8
α HCH	68.3	14.1
HCB	39.5	11.3
γ HCH	78.3	11.1
PCB 52	89.5	25.1
Aldrin	22.0	19.2
PCB 101	35.0	28.1
Dieldrin	60.7	15.1
Endrin	97.5	13.0
PP DDT	75.2	15.0
PCB 180	57.0	20.3
Cis Permethrin	63.5	20.4
Trans Permethrin	65.7	21.6

The recoveries are lower than those obtained using the manual systems and much lower than those reported in the manufacturers literature. The recoveries of the least polar compounds PCBs, Aldrin, HCB and HCBD are lower than the pesticides. Volatile non polar compounds give the poorest recoveries.

Previous extraction experience suggested that these poor recoveries were probably due to two main factors I) poor adsorption of the analytes from the water, and ii) poor elution of the analytes from the cartridge.

Non polar analytes such as Aldrin are most strongly adsorbed by the cartridge. Recovery of aldrin was much lower than similarly structured more polar compounds e.g. dieldrin. This may indicate low analyte elution of strongly held non polar compounds. Recoveries of low polarity compounds such as permethrin and dieldrin is 20-30% lower than the manufacturers indicated could be obtained with this system. Experience had shown these analytes are readily eluted from the cartridge with weakly polar solvents such as ethyl acetate. This suggests that these compounds were not being strongly held by the column and therefore were not retained during the extraction from the water sample. The results indicate that both analyte adsorption and analyte elution required further work.

The low recovery of very volatile compounds such as HCBD is greater than could be explained by either poor adsorption or elution. Because of their high volatility it is suggested that these compounds were being lost from the column during the drying step or during evaporation before the GC analysis.

All of these possible effects were investigated to maximise analyte recovery.

3.6.3.8 Optimisation of Analyte Retention

Phase Selection

One of the parameters which has a great impact on analyte retention is the phase of the cartridge. C2, C8 and C18 phases have all been successfully used for the extraction of organic compounds from aqueous matrices. The retention of organochlorine compounds on each of these phases was investigated.

Using methods similar to those described previously 6 1 L deionised water samples were spiked with a selection of organochlorine compounds. All of the samples were pre-treated by the addition of 50mls of IPA. Duplicate samples were analysed using C2, C8 and C18 cartridges on the Autotrace using the program listed in Table 70.

The mean recovery of the duplicate samples for each analyte are listed in Table 72.

Table 72 % Recoveries of Analytes Using Different Bonded Phases with the Autotrace Extraction System.

Compound	C2	C8	C18
HCBD	11.4	16.0	18.3
αHCH	54.7	65.7	66.8
HCB	30.9	40.8	36.8
γ HCH	65.8	74.5	75.5
PCB 52	76.3	81.0	75.8
Aldrin	18.0	20.3	20.8
PCB 101	34.4	36.2	32.1
Dieldrin	48.9	68.9	70.2
Endrin	85.3	91.2	93.4
PP DDT	65.4	79.3	75.1
PCB 180	55.2	60.2	50.0
Cis Permethrin	45.3	63.7	65.5
Trans Permethrin	47.8	67.8	66.1
Mean Recovery All Analytes	49.2	58.9	57.4

The C2 cartridge gives a slightly lower mean recovery than the C8 and C18 cartridges. This lower mean recovery suggests that the C2 cartridge does not adsorb the analytes from the aqueous solution as efficiently as the C8 and C18 cartridges. The mean recoveries of the C8 and C18 cartridges are very similar. The recovery of less polar compounds (e.g. PCB 180) which because of their low polarity are strongly adsorbed by all the phases is lower on the C18 cartridge than the C8 and C2 cartridge. This suggests that these analytes were so strongly adsorbed that they could not be quantitatively removed from the cartridge.

This confirmed the earlier conclusion that the C8 cartridge provided the best recoveries for a wide range of compounds.

Wetting Agent ,Temperature, and Salinity.

Manufacturers of all solid phase extraction cartridges recommend the addition of 5% methanol or IPA to the sample as a wetting agent. An investigation was carried out to determine whether the addition of a wetting agent improved recoveries.

Correspondence with a cartridge supplier (Crawford, pers comm.) indicated that US users (Burke, University of Arizona) of the Autotrace system had observed that the temperature of the sample had a significant effect on analyte retention when using a C18 phase. These users found that a sample extracted at 4°C showed an improved recovery of approximately 10% over samples extracted at 22°C. It was suggested by these users that this improvement was produced by lower temperatures reducing the rate at which the analytes diffused from the C18 phase into the silica pores. This lower rate reduced penetration making elution of these analytes easier. Temperature effects were investigated to determine whether improvement was observed when using a C8 phase.

As discussed for liquid-liquid extraction the addition of salt to the aqueous phase is common method of improving analyte recoveries in extraction processes. This was investigated to determine if a similar effect was observed for solid phase extraction.

Using methods similar to those described previously twelve 1L deionised water samples were spiked with a selection of organochlorine compounds. These samples were split into 4 batches of 3. Batch 1 was used without further modification, 50mls of IPA was added to each sample in Batch 2, the samples in batches 3 and 4 were stored in a refrigerator at 4°C overnight. Following removal from the refrigerator 50 mls of IPA was added to each sample in Batch 3, 50mls of IPA and 30 g of NaCl to each sample in batch 4. The samples were sequentially extracted using the Autotrace with the program listed in Table 70.

The mean recovery and RSD for each analyte are listed in Table 72.

The addition of wetting agent (50ml IPA) improves recoveries by approximately 10 %. The mean analyte recovery without wetting agent is 53.5 % and with wetting agent 63.3 %. The reduction in sample temperature does not show the 10% improvement in recoveries which had been reported by other workers. The reduction in sample temperature gives an approximate 20% reduction in mean recoveries. The addition of salt has a significant effect on recoveries, increasing the mean recovery by approx. 20% to 71%. The addition of salt and IPA as a wetting agent improves the recoveries for the majority of compounds to an efficiency comparable with the manual positive pressure based system. Exceptions are the recoveries for HCBD, HCB and aldrin. The recovery of HCBD is so poor (20%) that the system can not be considered quantitative for this compound.

Table 72 Investigation of Wetting Agent, Sample Temperature and sample salinity on SPE recovery.

Compound	22 °C Spiked Deionised Water		22 °C Spiked Deionised Water + 50ml IPA Wetting Agent		4 °C Spiked Deionised Water + 50ml IPA Wetting Agent		4 °C Spiked Deionised + 50ml IPA + 30g NaCl	
	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD
HCBD	8.94	9.85	17.5	23.1	19.78	16.6	21.8	16.8
α HCH	75.4	11.4	83.9	8.21	72.98	8.54	84.1	7.4
HCB	35.6	12.3	42.35	9.17	36.0	8.67	53.4	7.9
γ HCH	76.6	11.8	88.6	8.43	78.1	8.26	87.0	8.1
PCB 52	53.5	10.7	69.7	9.56	50.8	9.1	80.1	8.4
Aldrin	19.26	13.6	28.8	14.3	21.9	14.7	46.1	11.4
PCB 101	38.2	11.0	54.1	7.96	40.6	9.59	65.4	8.4
Dieldrin	57.2	11.1	67.8	8.39	50.9	8.14	76.2	8.6
Endrin	70.6	12.5	88.6	12.7	71.4	11.3	85.4	10.9
PP DDT	70.4	14.7	71.4	14.8	55.4	14.8	83.6	10.5
PCB 180	61.8	11.61	70.9	7.85	48.9	7.9	81.3	7.6
Cis Permethrin	62.2	14.7	71.4	9.54	49.3	8.6	79.3	8.4
Trans Permethrin	65.9	12.58	67.2	9.16	50.5	8.8	79.3	8.5
Mean Recovery All Analytes	53.5	-	63.3	-	49.7	-	71.0	-

Extract Drying/ Evaporation

It was observed that the extract obtained from the Autotrace was cloudy, investigation of this revealed that rather than the expected 12ml total volume (2*3ml Ethyl Acetate, 2*3ml Hexane) the extract had a volume of 14-16mls. It was speculated that this increase in volume might be residual salty water from the cartridge and the sample lines being eluted from the system. Further examination of the extracts previously analysed by GC on earlier tests revealed that these extracts even after drying and concentration also contained small droplets of water. Earlier investigations on evaporation and drying steps had focused on and determined optimum conditions for hexane extracts. Tests were carried out to determine if this residual water was contributing to the poor recoveries of HCBD, HCB and aldrin.

Combinations of 30 % saline solution / ethyl acetate / hexane were spiked with organochlorine compounds and taken through the drying concentration steps which had been used in the previous tests. The dry 1 ml extracts were analysed by GC/ECD and the % Recovery of each compound determined.

The % recovery for each of the analytes is listed in Table 73.

Table 73 Investigation of Extract Drying / Evaporation on Analyte Recovery.

	12 ml Hexane	6ml Ethyl Acetate 6 ml Hexane	6ml Ethyl Acetate 6 ml Hexane 1ml Saline Sln	6ml Ethyl Acetate 6 ml Hexane 2ml Saline Sln	6ml Ethyl Acetate 6 ml Hexane 3ml Saline Sln
HCBD	92	57	54	56	52
α HCH	96	84	84	84	80
HCB	97	86	84	81	81
γ HCH	97	81	77	76	76
PCB 52	97	81	82	80	91
Aldrin	98	83	82	81	80
PCB 101	96	84	84	87	87
Dieldrin	97	86	85	86	84
Endrin	106	91	90	85	82
PP DDT	101	88	89	86	84
PCB 180	98	91	90	89	91
Cis Permethrin	97	94	94	89	90
Trans Permethrin	98	91	91	91	91
Mean Recovery All Analytes	98	84	84	82	82

The recoveries for the hexane extract are excellent and very comparable with the TurboVap optimisation tests. The recoveries obtained from the combinations of hexane / ethyl acetate, hexane / ethyl acetate / saline solution are significantly lower than those obtained from hexane. These combinations took considerably longer to evaporate. No significant differences are observed both in the recoveries of analytes or length of evaporation time between the hexane / ethyl acetate, and hexane / ethyl acetate / saline solution combinations.

The recoveries of α HCH, γ HCH and dieldrin from the evaporation and drying of the hexane / ethyl acetate combination are similar to the recoveries obtained for these compounds from the complete extraction procedure in section 3.6.3.7. The losses of all the analytes during the drying and evaporation steps represents a significant portion of the losses observed in the complete extraction procedure.

Ethyl acetate has a boiling point of 77 °C this is slightly higher than hexane and may explain the poor recoveries obtained when evaporating hexane / ethyl acetate mixtures.

Two possibilities were considered to improve recovery from evaporation a) New optimum evaporation conditions b) replacement of ethyl acetate by a solvent of similar polarity with boiling point closer to that of hexane.

Different evaporation conditions for various solvent combinations was considered not to be a practicable alternative. Multiple sets of optimum conditions would require settings on the TurboVap evaporator to be frequently changed and re-calibrated. This would take considerable time at each change over step and reduce the flexibility of the evaporator. Therefore it was considered more practicable to use an alternative solvent to ethyl acetate.

Replacement Polar Solvent.

The alternative solvent to ethyl acetate should posses similar chemical properties including a low boiling point, similar polarity, good evaporation properties, be safe to handle and be readily available in high purity at a reasonable price. Methyl tert butyl ether (MTBE) and acetone were both considered as possible replacement solvents.

Solutions of 50% MTBE / hexane and 50% acetone / hexane were spiked with organochlorine compounds and taken through the drying concentration procedure. The extracts were analysed by GC/ECD and the % recovery of each compound determined.

The % recovery for each of the analytes is listed in Table 74.

Table 74 Investigation of Replacement Polar Solvent for Analyte Elution.

	50 % MTBE / Hexane	50% Acetone / Hexane
HCBD	90	91
α HCH	96	97
HCB	97	95
γ HCH	97	101
PCB 52	98	96
Aldrin	97	97
PCB 101	95	94
Dieldrin	99	98
Endrin	101	106
PP DDT	98	97
PCB 180	97	102
Cis Permethrin	96	101
Trans Permethrin	98	99

Both of these solvent combinations gave excellent recoveries using the drying / evaporation procedure which had been optimised for hexane.

Elution Properties of MTBE / hexane and acetone / hexane

In order to determine the combination of solvents giving the highest elution recoveries spiked deionised water samples were solid phase extracted and eluted with MTBE / hexane or acetone / hexane.

Using a similar method to that described previously twelve 1 L deionised water samples were spiked with organochlorine compounds. These spiked samples were pre-treated by the addition of 50mls of IPA and 30 g of Sodium Chloride . The samples were mixed thoroughly and processed on the Autotrace using the program listed in Table 75, where SOLVENT 2 is MTBE for the first six samples and acetone for the remaining six samples.

Table 75AUTOTRACE Program.

AUTOTRACE EXTRACTION PROCEDURE
Estimated Time For samples 78.9 minutes

Step 1 : Process 6 samples using the following procedure:
Step 2 : Wash syringe with 10ml SOLVENT 2
Step 3 : Wash syringe with 10ml IPA
Step 4 : Condition column with 10ml IPA into solvent WASTE
Step 5 : Condition column with 10ml SOLVENT 2 into solvent WASTE
Step 6 : Condition column with 10ml IPA into solvent WASTE
Step 7 : Condition column with 10ml DEINOISED H2O into solvent AQUEOUS WASTE
Step 8 : Load 1100ml of sample onto column
Step 9 : Rinse column with 10 ml of DEIONISED H2O into AQUEOUS WASTE
Step 10 : Dry column with gas for 2min
Step 11 : Wash syringe with 5ml SOLVENT 2
Step 12 : Wash syringe with 5ml SOLVENT 2
Step 13 : Soak and Collect 3 ml fraction using SOLVENT 2
Step 14 : Collect 3 ml fraction into sample tube using SOLVENT 2
Step 15 : Soak and Collect 3 ml fraction using HEXANE
Step 16 : Collect 3 ml fraction into sample tube using HEXANE
Step 17 : END

SETUP PARAMETERS

FLOW RATES		SPE PARAMETERS	
Cond Flow :	40 ml/min	Push Delay :	5 sec
Load Flow :	30 ml/min	Air Factor :	1
Rinse Flow	40 ml/min	Autowash Vol :	0.00 ml
Elute Flow :	20 ml/min		
Cond Air Push :	40 ml/min	WORKSTATION PARAMETERS	
Rinse Air Push :	40 ml/min	Max. Elution Vol:	20.0 ml
Elute Air Push :	20ml/min	Exhaust Fan on:	N
		Beeper on:	Y

The % recovery for each of the analytes is listed in Table 76.

Table 76 Investigation of Elution Solvent

	MTBE / Hexane		Acetone / Hexane	
Compound	Mean	RSD	Mean	RSD
HCBD	7.5	83	42.3	12.5
α HCH	21.6	21	86.8	6.8
HCB	34.5	25	73.6	7.6
γ HCH	21.7	41	88.6	7.9
PCB 52	36.0	33	84.2	8.2
Aldrin	35.7	14	76.3	8.4
PCB 101	40.9	28	78.9	8.4
Dieldrin	43.8	32	89.6	8.9
Endrin	56.4	68	92.3	12.6
PP DDT	42.9	18	88.1	14.5
PCB 180	37.8	19	78.0	5.4
Cis Permethrin	38.6	29	91.7	8.0
Trans Permethrin	37.6	34	92.6	7.6
Mean Recovery of all analytes	35.0	-	81.8	-

The elution recoveries for MTBE / hexane are very poor. The recoveries are lower and more variable than obtained with ethyl acetate / hexane as an elution solvent. The extremely poor recoveries and high variability may be due to the poor aqueous solubility of MTBE. It has been reported by other workers (Van Horne, 1985) that trace quantities of residual water are trapped in and around the C18 phase, these water molecules may repel the MTBE preventing it reaching the analytes and hence lowering the recoveries. MTBE/hexane is not suitable as an elution solvent. The recoveries for elution with acetone / hexane are excellent. The recoveries are quantitative for all the compounds except HCBD, and approach the claims in the manufacturer application notes. Although elution with acetone / hexane was a significant improvement a large amount of water was still obtained in the final extract. It was extremely difficult to remove all of this water by filtering the extract through anhydrous sodium sulphate and as a result the final 1 ml extract which was presented to the GC often contained small droplets of water. This was not an acceptable situation as injection of water onto the capillary column would damage the column and send the electron capture detector off scale. A method of producing less carry-over of water from the cartridge or a more efficient drying process was required.

Investigation of C8 Column Drying

To minimise the retention of water in the C8 extraction cartridge a series of spiked deionised water samples were solid phase extracted, the length of time the cartridges were dried for was varied to determine the effects on the moisture content of the extract and the recovery of the analytes.

Using the method described previously 1 L deionised water samples were spiked with organochlorine compounds. These spiked samples were pre-treated by the addition of 50mls of IPA and 30 g of Sodium Chloride . The samples were mixed thoroughly and processed on the Autotrace. The program listed in Table 75 with acetone and hexane as elution solvents was used to process the samples, however, the dry column stage was sequentially increased from 2 - 16 mins.

Increasing the drying time did not have a significant effect on recovery for the majority of analytes. For a drying of 16 min a significant decrease in HCBD recovery is observed. Increasing the drying time from 2 to 4min did significantly reduce the amount of water present in the extract. Further increases in the drying time from 4 to 16min does not decrease the amount of water in the extract. 4 min was selected as the optimum drying time. Even after drying with anhydrous sodium sulphate all of the extracts contained small amounts of residual water.

The % recovery for each of the analytes is listed in Table 77.

Table 77 Effect of Drying Time on Mean analyte Recovery

	Drying Time			
Compound	2 mins	4 mins	8mins	16 mins
HCBD	42.3	44.3	40.9	28.6
α HCH	86.8	91.4	88.6	81.2
HCB	73.6	72.0	70.4	54.3
γ HCH	88.6	90.1	85.9	82.4
PCB 52	84.2	84.6	80.6	77.6
Aldrin	76.3	79.8	81.5	71.4
PCB 101	78.9	77.5	80.9	74.2
Dieldrin	89.6	88.4	87.5	90.5
Endrin	92.3	90.6	100.5	98.0
PP DDT	88.1	96.7	91.4	84.7
PCB 180	78.0	84.1	92.4	88.9
Cis Permethrin	91.7	94.8	88.9	90.8
Trans Permethrin	92.6	90.2	94.7	94.2
Mean Recovery of all analytes %	81.8	83.4	83.4	78.2
Volume of Water in extract (mls)	3	0.5	0.5	0.5

Acetone and water demonstrate a great affinity towards each other and are completely miscible. The removal of water from 50% acetone / hexane mixtures using traditional chemical desiccants such as anhydrous sodium sulphate was only partially successful.
Alternative methods of water removal such as the addition of excess saline solution and back extraction with hexane, use of a stronger desiccant, and freezing out were considered. The time required, and the probability of interfering with the target analytes resulted in the rejection of all of these methods.
The removal of water from hexane extracts using anhydrous sodium sulphate during the liquid - liquid extraction development work was extremely successful. It was speculated that the removal of the water using anhydrous sodium sulphate would be more efficient if there was less acetone present in the extract.

Tests were carried out to determine the minimum quantity of acetone required to remove all of the water from the cartridge and hence allow quantitative recovery of the analytes.

Using the method described previously 1 L deionised water samples were spiked with organochlorine compounds. These spiked samples were pre-treated by the addition of 50mls of IPA and 30 g of Sodium Chloride . The samples were mixed thoroughly and processed on the Autotrace. The program listed in Table 75 was used to process the samples. The optimum drying time of 4 mins was used to dry the cartridges. A variety of acetone/ hexane mixtures were used as elution solvents. The elution conditions investigated are summarised in Table 78.

Table 78 Autotrace Elution Conditions

Test	Elution Conditions
Conditions A	Soak and Collect 3ml 25% Acetone / Hexane Collect 3ml 25 % Acetone / Hexane Soak and Collect 3ml Hexane Collect 3ml Hexane
Conditions B	Soak and Collect 3ml 50% Acetone / Hexane Collect 3ml 50 % Acetone / Hexane Soak and Collect 3ml Hexane Collect 3ml Hexane
Conditions C	Soak and Collect 3ml 75% Acetone / Hexane Collect 3ml 75 % Acetone / Hexane Soak and Collect 3ml Hexane Collect 3ml Hexane

The % recovery for each of the analytes is listed below in Table 79.

	% Recovery		
Compound	Conditions A	Conditions B	Conditions C
HCBD	47.8	62.3	51.6
α HCH	71.3	89.7	85.6
HCB	56.8	77.9	72.1
γ HCH	78.6	92.6	88.3
PCB 52	74.9	88.6	81.6
Aldrin	59.6	84.7	82.3
PCB 101	78.9	86.9	84.9
Dieldrin	78.9	90.4	89.6
Endrin	82.3	96.7	97.3
PP DDT	73.6	101.3	92.6
PCB 180	77.2	92.7	95.4
Cis Permethrin	74.9	95.7	90.1
Trans Permethrin	73.0	97.3	91.6
Mean Recovery of all analytes %	71.4	89.0	84.85

No water droplets were observed in the extracts prior to GC analysis. Therefore, reducing the % of Acetone in the extract did improve the efficiency of the drying process. This resulted in improved recoveries of HCBD in all of the test conditions. Test conditions A produced lower recoveries for the majority of analytes indicating that residual water remained in the cartridge preventing recovery of a small % of the test analytes. Elution with 50% acetone / hexane followed by elution with hexane was selected as the optimum elution conditions. The improved efficiency of the drying process improves the recoveries for HCBD. However, there is still sufficient acetone to remove residual water on the cartridge and ensure complete recovery of the target analytes.

3.6.4 Optimisation of Extraction Methods for Solid Samples.

Section 2.4.2 outlines a variety of extraction methods which have been used for the extraction of solid samples. From this review of analytical methods it is clear that Soxhlet and SFE are the two techniques most appropriate for the extraction of solid matrices. The equipment necessary to undertake SFE was not available for this study. In this section the use of soxhlet extraction for the recovery of a range of contaminants from dried sediment and biota was investigated.

3.6.4.1 Preparation of Test Matrices

As indicated in section 3.6 the most efficient method of comparing extraction efficiency is to compare the concentrations obtained from the analysis of " naturally contaminated " samples. No one matrix could be considered as representative of all sample types. To allow optimisation of all the range of matrices which would be encountered in environmental studies two test matrices were prepared. Large quantities were prepared in anticipation of the use of these matrices as internal laboratory reference materials (LRM).

Test Matrix 1 : Sediment collected from a variety of locations within the Clyde Estuary was selected as representative of sediment matrices. A number of authors (Halcrow et al .,1974; Tyler et al .,1994) have indicated that Clyde Estuary sediment contains elevated concentrations of organochlorine compounds, this makes it suitable for both a test recovery matrix and a LRM. The sediments were freeze dried, lightly ground using a mortar and pestle and sieved using a 63μm brass sieve. The less than 63 μm portion was thoroughly mixed and used as the reference material. After preparation the material was stored in amber jar within a desiccator.

Test Matrix 2 : Mussels *Mytilus Edulis* were selected as representing low fat tissue samples. Mussel homogenate was freeze dried, macerated, ground and mixed to give a uniform powder. After preparation the material was stored in an amber jar within a desiccator. The mussels were sampled from the Ythan Estuary (North East Scotland) an area known to have high levels of PCB residues.

The variables which effect the efficiency of a soxhlet extraction are i) the length of time of the extraction and ii) the choice of extraction solvent. Tests were undertaken to investigate the effects of both these variables on extraction efficiency.

3.6.4.2 Investigation of Soxhlet Extraction Conditions.

Duplicate portions of the sediment (5g) and mussel homogenates (2g) were soxhlet extracted using the conditions listed in Table 80. Approximately 10g of Copper powder cleaned as described in section 3.5.1 was added to the extraction solvent of the sediment samples prior to beginning extraction.

Table 80 Soxhlet Extraction Conditions

Extraction Solvent	Extraction Time (Hours)
Hexane	7
50 % Hexane / MTBE	7
MTBE	7
DCM	7
Hexane	14
50 % Hexane / MTBE	14
MTBE	14
DCM	14

The resultant extracts were reduced in volume to 1 ml by TurboVap concentration. The reduced volume extract was split into two equal portions. Total extractable material was determined gravimetrically on the first portion using the procedure described in section 3.4.2.2. The second portion of biota extracts were cleaned up using the Neutral Alumina procedure described in section 3.4.4.2. The second portion of sediment extracts were cleaned-up using the Silver-Nitrate Alumina procedure described in 3.4.4.3. The cleaned-up sediment and biota extracts were fractionated using the silica gel separation described in section 3.4.1. The analytes were determined by GC/ECD analysis using the conditions described in section 3.2. The concentration of target analytes determined in the matrices expressed in ng/g are listed in Tables 81 and 82.

The extraction efficiencies of the various solvent combinations and extraction times were compared for the analytes for which positive results were obtained (PCBs, PP DDE, PP DDD, PP DDT and Dieldrin). The mean result of each analyte for every experimental condition was calculated, these mean results were compared using a paired t-Test (Miller & Miller, 1988). In this the difference between the mean analyte results for two methods was calculated for each analyte. The null hypothesis that there are no significant differences in the mean concentrations for each analyte determined by the two methods was adopted. If this hypothesis is true the mean of the differences will be zero. Whether the mean of the differences differs significantly from zero can be tested using the following equation

$$t=\chi_d\sqrt{n}/s_d$$

where : t has n degrees of freedom and

χ_d = the mean difference of all analytes,

n = the number of degrees of freedom,

s_s = the standard deviation of the differences between the analytes.

The t values for the comparison of the different methods is listed in table 83.

Table 81 Optimisation of Sediment Extraction Conditions.

Extraction Time	7 Hours						14 Hours					
Extraction Solvent	50 % Hexane MTBE		MTBE		DCM		50% Hexane MTBE		MTBE		DCM	
Compound	Concentration ng/g dry weight											
HCBD	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
HCB	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PCB 28	15.8	13.1	17.7	16.4	16.4	16.1	16.4	15.8	18.3	16.7	19.4	16.1
PCB 52	18.7	19.2	19.9	21.1	20.7	19.8	19.8	20.1	19.0	20.3	19.4	19.9
Aldrin	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
OP DDE	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PCB 101	8.5	8.1	9.5	8.8	9.0	8.6	8.6	7.6	8.3	7.2	8.4	9.0
PP DDE	7.1	6.8	7.2	7.4	6.4	7.5	7.1	7.3	7.4	7.6	7.6	6.9
PCB 118	10.1	10.9	9.9	11.1	11.2	14.1	13.8	14.0	13.6	13.8	13.2	14.3
PCB 153	11.0	10.9	11.3	11.8	10.9	12.1	11.1	11.3	12.4	11.6	11.2	12.3
PCB 138	10.1	10.6	11.8	10.4	10.4	11.2	10.0	11.1	12.1	11.0	9.8	10.9
PCB 180	6.4	5.9	6.0	7.4	7.9	8.6	6.4	7.1	7.2	7.3	7.4	7.0
α HCH	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β HCH	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
γ HCH	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Endo A	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Dieldrin	4.4	4.9	7.2	7.4	7.5	7.6	6.4	6.2	7.0	6.9	7.8	7.2
Endrin	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Endo B	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PP DDD	7.2	7.4	8.4	8.9	9.1	8.9	8.6	7.9	8.6	8.4	8.8	8.3
OP DDT	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PP DDT	1.8	2.6	2.4	2.0	3.2	2.3	2.4	2.9	2.7	1.9	2.1	2.6
Cis Permethrin	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
Trans Permethrin	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
Total Extractable Material	32	32	34	36	32	35	33	32	35	34	33	35

Table 82 Optimisation of Biota Extraction Conditions

Extraction Time	7 Hours						14 Hours					
Extraction Solvent	50 % Hexane MTBE		MTBE		DCM		50% Hexane MTBE		MTBE		DCM	
Compound	Concentration ng/g dry weight											
HCBD	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
HCB	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PCB 28	2.1	2.3	2.4	2.5	2.4	2.4	2.0	2.2	2.4	2.3	2.5	2.2
PCB 52	22.3	22.0	22.4	22.5	22.3	22.1	22.1	22.2	22.7	21.0	22.4	22.0
Aldrin	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
OP DDE	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PCB 101	70.1	74.6	72.3	77.2	71.3	70.9	74.6	72.3	75.6	74.6	72.8	73.9
PP DDE	4.2	5.1	5.0	4.7	5.3	5.6	4.7	5.0	5.1	5.6	4.9	4.7
PCB 118	64.2	63.6	62.1	65.1	63.8	66.0	61.1	66.5	66.4	65.7	63.4	64.7
PCB 153	62.8	66.4	62.3	64.1	63.1	62.5	63.7	60.1	65.3	64.2	64.5	62.9
PCB 138	91.3	97.3	92.3	88.6	88.3	96.8	91.6	96.4	92.4	97.6	91.4	93.4
PCB 180	5.7	6.3	6.0	6.1	6.4	6.0	6.0	6.3	6.4	6.2	6.1	6.0
αHCH	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β HCH	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
γ HCH	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Endo A	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Dieldrin	3.4	3.1	3.4	3.2	3.2	3.1	3.4	3.3	3.6	3.2	3.4	3.7
Endrin	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Endo B	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PP DDD	7.7	7.4	7.6	7.0	8.1	7.6	7.7	8.2	8.0	7.9	7.7	8.1
OP DDT	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PP DDT	2.1	2.1	2.0	2.3	2.4	2.0	2.1	2.2	1.9	2.4	2.1	2.1
Cis Permethrin	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
Trans Permethrin	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
Total Extractable Material	76	71	75	73	73	74	73	73	74	76	73	72

Table 83 t values for Comparison of Sediment Extraction Methods

Methods	7 Hour 50% MTBE/Hexane t 7 Hour MTBE	7 Hour 50% MTBE/Hexane to 7 Hour DCM	7 Hour MTBE to 7 Hour DCM	7 Hour 50% MTBE/Hexane to 14 Hour 50% MTBE/Hexane	7 Hour MTBE to 14 HourMTBE	7 Hour DCM to 14 Hour DCM
t	3.761	5.075	0.366	0.552	0.467	0.217
Significance	**	**	NS	NS	NS	NS

* t value significant at 0.05 probability level. Evidence of a difference in extraction efficiencies.

**t value significant at 0.01 probability level. Very strong evidence of a difference in extraction efficiencies.

NS not significant. No evidence of a difference in extraction efficiencies.

Critical t values from Lide (1993).

For sediments the t value obtained when comparing 7 Hour 50% MTBE / hexane extractions to 7 Hour MTBE and DCM extractions is greater than the critical value of t at both the 0.05 and 0.01 probability level (2.201, and 3.106). Therefore, the null hypothesis is rejected indicating that the extraction efficiency of 50% MTBE/ hexane is significantly different from the efficiency of MTBE and DCM. The mean analyte concentrations obtained when using 50% MTBE / hexane extraction are lower than those obtained using MTBE or DCM as extraction solvents. This indicates that a 7 Hour 50% MTBE / hexane does not extract all of the analytes present in the sample. These results confirm the sediment acts as a chromatographic material. The 50% MTBE / hexane extraction solvent is not polar enough to remove all of the analytes form the mineral components in the sediments which are acting as a normal phase chromatographic material.

The t values obtained when comparing the 7 and 14 hour extractions in all 3 solvent combinations are lower than the critical value at both the 0.05 and 0.01 probability levels. Therefore, the null hypothesis is retained indicating that the extraction efficiencies do not significantly differ. This indicates that in a 7 hour extraction period the tested solvent combinations will have reached their extraction limit and increasing the extraction time will not improve the extraction efficiency.

7 hour soxhlet extractions with either MTBE or DCM are suitable for the extraction of the target analytes from sediments. As both solvents showed no differences in extraction efficiency other criteria were used to select the solvent system for routine use. For the reasons previously indicated DCM is not acceptable as a solvent for routine use. MTBE is a highly flammable solvent, its use with a electrically heated soxhlet extractor does present a slight fire hazard. This risk was considered less significant than the risk associated from prolonged exposure to a suspected carcinogen.

Therefore, a 7 Hour soxhlet extraction with MTBE was chosen as the optimum conditions for the extraction of sediment samples.

For biota samples the analytes for which positive results were obtained were statistically analysed using the methods described previously for the analysis of sediment extraction efficiencies. The results of this statistical analysis are presented in Table 84.

Table 84 t values for Comparison of Biota Extraction Methods

Methods	7 Hour 50% MTBE/Hexane to 7 Hour MTBE	7 Hour 50% MTBE/Hexane to 7 Hour DCM	7 Hour MTBE to 7 Hour DCM	7 Hour 50% MTBE/Hexane to 14 Hour 50% MTBE/Hexane	7 Hour MTBE to 14 HourMTBE	7 Hour DCM to 14 Hour DCM
t	-0.401	-0.707	-0.053	-0.547	2.194	0.217
Significance	NS	NS	NS	NS	NS	NS

* t value significant at 0.05 probability level. Evidence of a difference in extraction efficiencies.
** t value significant at 0.01 probability level. Very strong evidence of a difference in extraction efficiencies.
NS not significant. No evidence of a difference in extraction efficiencies.
Critical t values from Lide (1993).

The null hypothesis is retained in all of the above tests. This indicates no significant differences in the extraction efficiencies of the test solvent compositions or in the length of time of extraction. In contrast to the results obtained for sediments 50 % MTBE / hexane is a suitable extraction solvent. Biota do not contain minerals which will strongly retain the analytes by normal phase mechanisms, in biota the target analytes will be associated with free lipids (e.g. neutral lipids, cholesterol esters and wax esters) and bound lipids (e.g. phospholipids) (de Boer, 1988). The medium polarity 50% MTBE / hexane is a strong enough solvent to ensure complete recovery of the target analytes from this matrix.

Although all 8 tested extraction procedures were suitable for use, for simplicity and comparability with the optimum sediments procedure a 7 Hour MTBE soxhlet extraction was selected as the optimum procedure for the extraction of biota samples.

3.7 Optimum Analytical Procedures

As indicated in section 3.1 the aim of the method development was to optimise a series of modular based analytical protocols. Each module to be optimised for a specific function. For a particular sample or sample type optimised modules could be selected to tackle the specific requirements of that sample. The modular nature would minimise the amount of staff training and maximise the usage of analytical space and equipment, by rather than having areas and equipment dedicated to a particular sample type they would be allocated to modules, these modules to be used across a variety of matrix types.

The following section summarises the developed modules, and sets out flow diagrams indicating how these modules may be linked for the routine determination of the target analytes in 5 commonly encountered sample types.

Module 1: **Hexane Liquid - Liquid Extraction** - Double extraction of a 1 litre sample with 50 mls of Hexane. The extraction is carried out in the sample collection container. The extracts are dried by passing through anhydrous sodium sulphate.

Module 2: **Solid Phase Extraction** - using a 1g C8 End Capped Isolute cartridge with a 6ml reservoir volume. Samples are pre-treated with 50mls of IPA, and 30g of salt. The samples are processed using a Zymark Autotrace operated as described in the manufacturers manuals. The optimised program is summarised below:

Parameter	Optimised Conditions
Cartridge Activation	10 mls of Hexane, 10 mls of IPA, 10 mls of Hexane, 10 mls IPA, 10 mls Deionised Water.
Sample Flow Rate	30 ml/min
Cartridge Drying	Air for 4mins
Cartridge Elution	3 mls 50 % Acetone / Hexane Soak and Collect, 3 mls 50 % Acetone / Hexane Collect, 3mls Hexane Soak and Collect, 3ml Hexane Collect.
Elution Rate	20 ml/min

The sample container is rinsed with 20mls of Hexane, this is combined with the eluate from the Autotrace, and the combined extracts dried by passing through anhydrous sodium sulphate.

Module 3: **Soxhlet Extraction -** Dried samples are soxhlet extracted for at least 7 Hours using MTBE as the extraction solvent.

Module 4: **Sulphur Removal** - using tetra butyl ammonium sulphate.

Module 5: **Addition of Surrogate Standards** - Using a glass microsyringe 40pg of PCB 53 and ε HCH are added to the extracts.

Module 6 : **Extract Concentration** - Extracts are concentrated using a TurboVap concentrator. The instrument is operated in accordance with the manufacturers operating instructions. The optimised operating conditions are summarised below.

Model	Zymark TurboVap Evaporator
Gas Pressure	15 psi
Keeper	Iso-Octane
Bath Temperature	25-30 °C
Usage	Stop evaporation regularly and rinse tube wall with hexane.

Module 7: **Florisil Clean-Up** - using 2g Isolute florisil cartridges with a 6ml reservoir volume, the sample extract is quantitatively loaded on to the cartridge, the cartridge is eluted with 20 mls of 10% MTBE in Hexane.

Module 8: **Neutral Alumina Clean-Up** - using columns containing 6g of Alumina which has been activated by heating in a furnace at 500 °C for 4 hours and then deactivated by the addition of 10 % w/w water. The sample extract is quantitatively transferred to the column and analytes eluted with 100mls of Hexane.

Module 9: **Silver Nitrate Impregnated Alumina Clean-Up** - using columns containing 6g of Alumina activated by heating to 500 °C for 4 hours, and subsequently deactivated by the addition of 10 % w/w of a 75% w/v silver nitrate solution. The sample extract is quantitatively transferred to the column and analytes eluted with 100mls of Hexane.

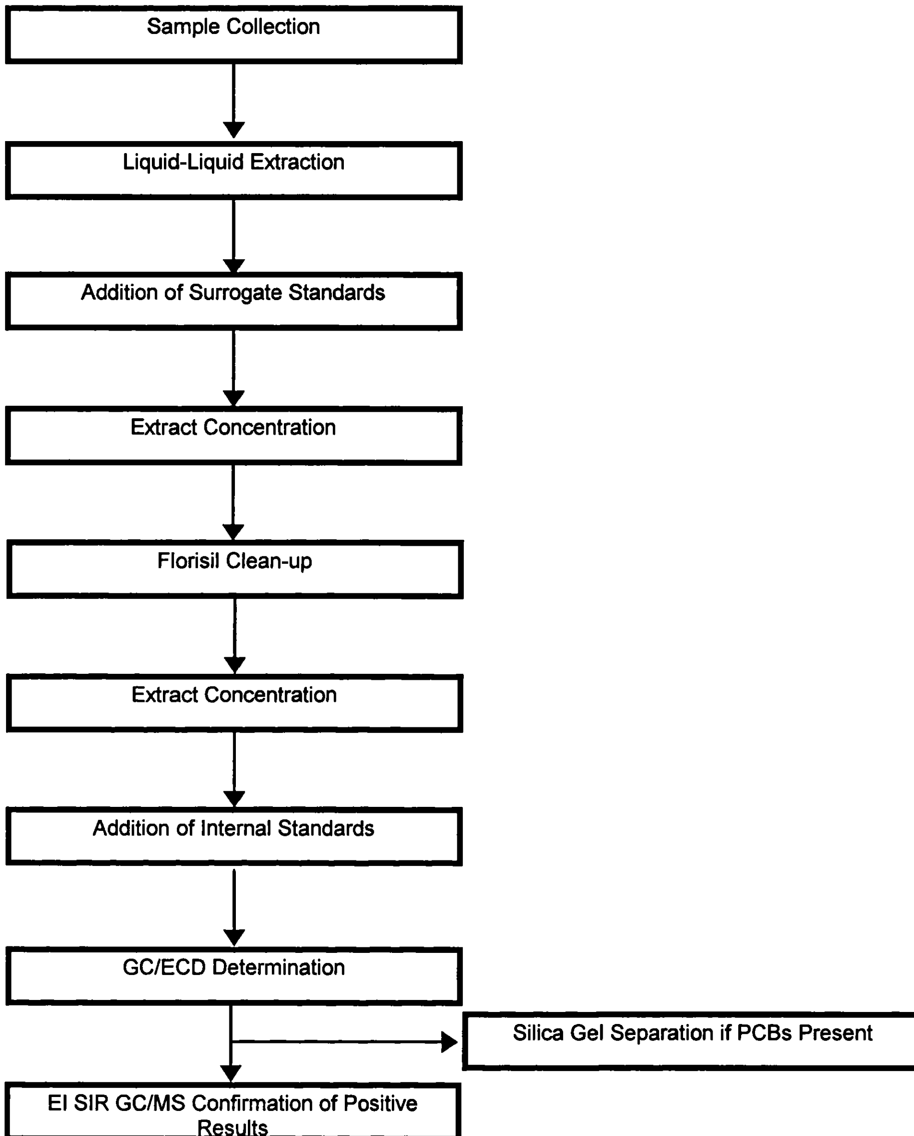
Module 10: **Silica Gel Separation** - using columns containing 2g of Silica Gel which has been activated by heating to 500 °C for 4 hours. The sample extract is quantitatively transferred to the column. The column is eluted with 10mls of Hexane followed by 25 mls of 20% MTBE in Hexane. The first 8mls eluted from the column contains PCBs and non polar analytes, the remaining 27 mls eluted from the column contains the remaining analytes.

Module 11 : **Internal Standard Addition** - Using a glass micro syringe 40 pg of THE, PCB112, PCB 198 and DCBP are added to the extract.

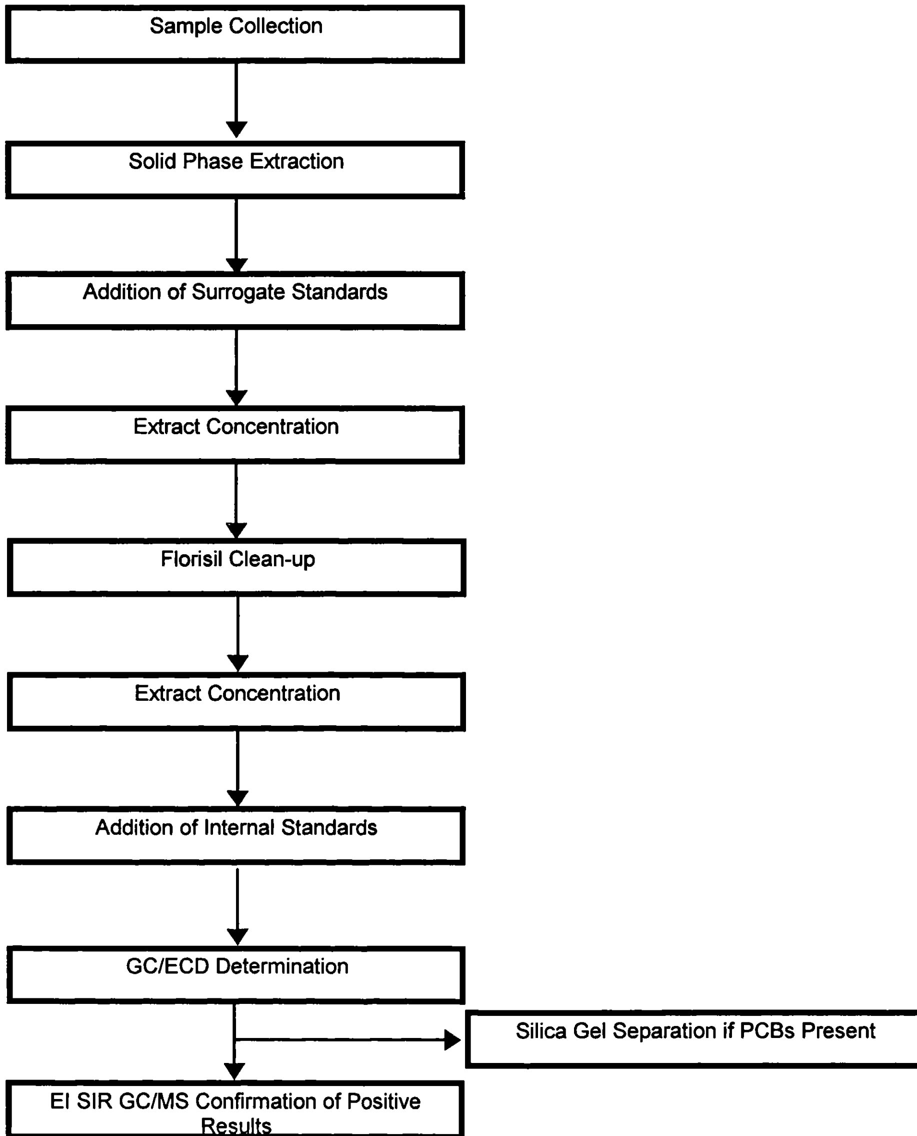
Module 12 : **GC/ECD Determination**

Module 13 : **EI SIR GC/MS Confirmation.**

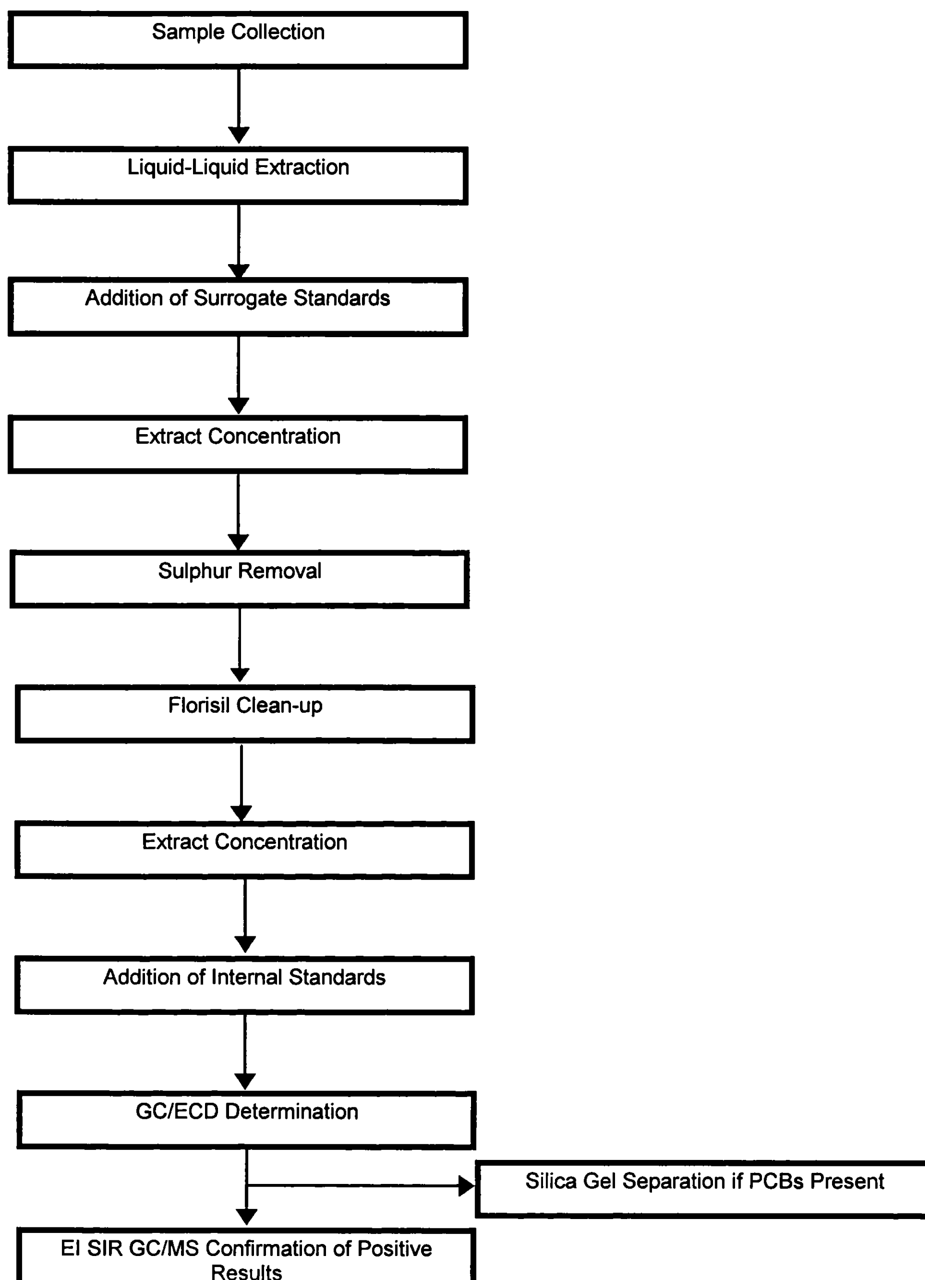
3.7.1 Method A :Determination in River Waters by Liquid-Liquid Extraction



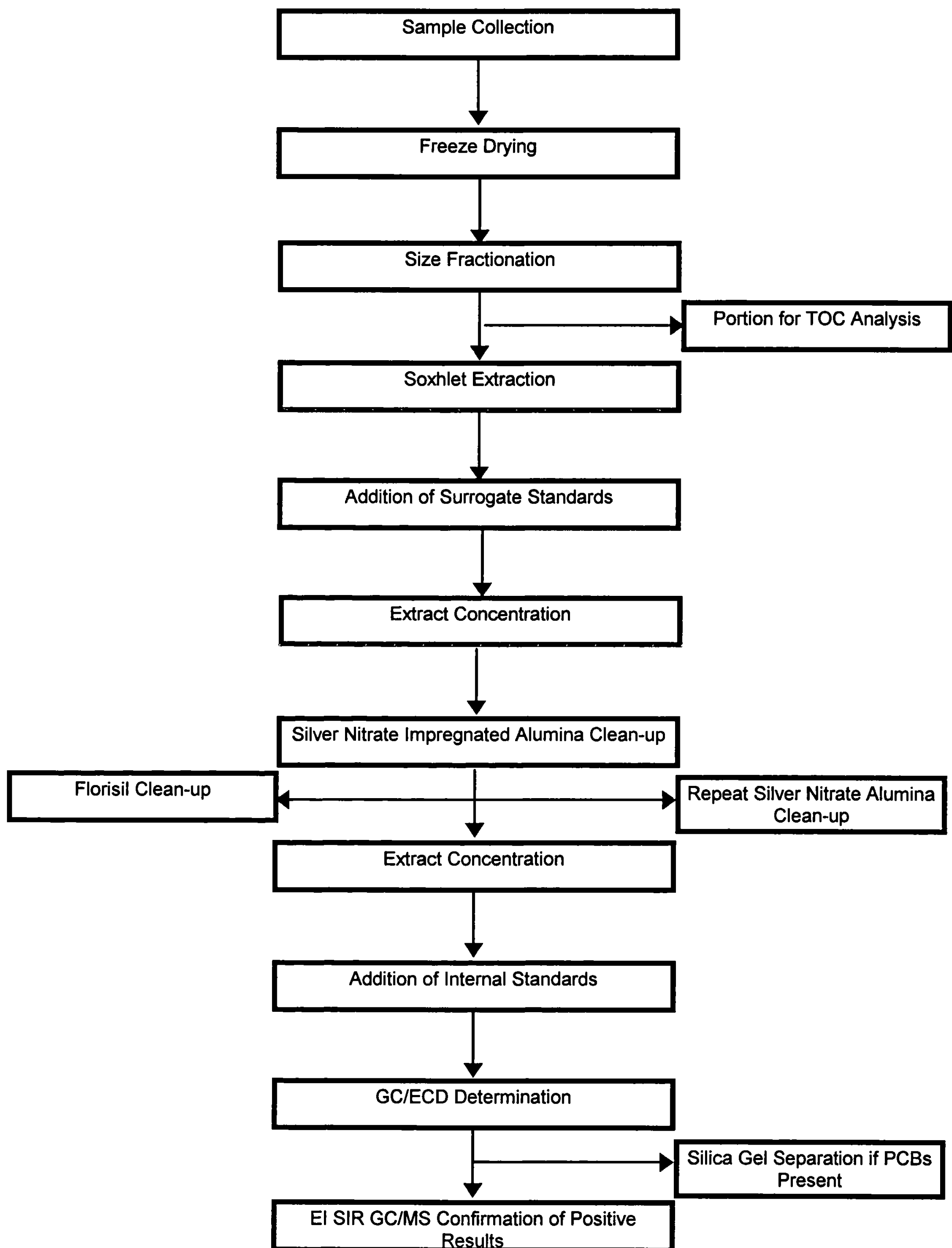
3.7.2 Method B :Determination in River Waters by Solid Phase Extraction



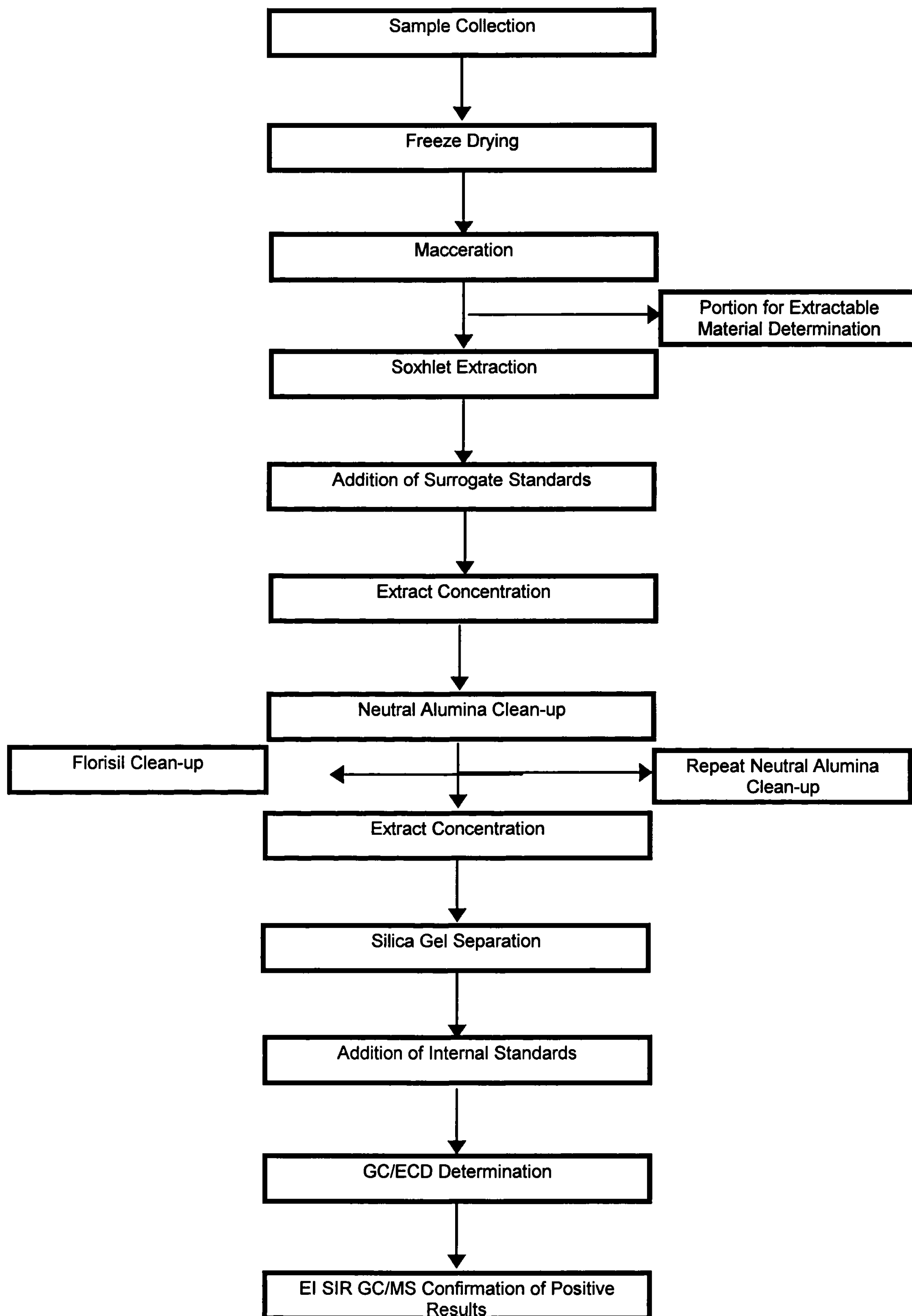
3.7.3 Method C: Determination in Effluents by Liquid-Liquid Extraction



3.7.4 Method D: Determination in Sediments



3.7.5 Method E: Determination in Biota



3.8 Calculation of Results and Routine Quality Control.

3.8.1 Calculation of Results

The raw concentration of an analyte in an extract is calculated from the calibration procedures described in section 3.2.4. This concentration is corrected for losses of analytes during the sample preparation and for the amount of sample analysed using the following general equation.

$$R = ((100/\%Y)*Y)/Z$$

Where	R =	Concentration of Analyte in sample
	Y =	Raw concentration (ug/l) of Analyte in extract.
	%Y =	% Recovery of compound Y determined from the Extraction/Process Recovery Standard
	Z =	The amount of sample analysed in litres (Aqueous samples) or Kilograms (Solid Matrices)

Detailed description of the use of Extraction and Process recovery standards

3.8.2 Routine Quality Control

The aim of routine quality control procedures are to provide and maintain confidence that the analytical procedure satisfies the predetermined analytical method performance characteristics with respect to accuracy and precision. Routinely a number of check measurements is made to ensure that analysis of the required quality is maintained. Three main areas have been identified as important in the control of the analytical procedures : 1) GC system Performance, 2) Calibration, 3) Control of the sample preparation.

3.8.2.1 GC System Performance

The validity of the analysis is dependent on the quality of the chromatographic analysis. Four critical areas are visually checked on all chromatograms.

a. Peak Resolution

Environmental samples contain complex mixtures of compounds whose resolution requires efficient narrow bore capillary columns. Acceptable resolution is assessed by the separation of PCB 101and OP DDE which must be at least 70 % resolved before the resolution can be considered adequate.

b. Peak Shape

Peaks should not tail. Peak width at half height should be less than 6 secs.

c. Baseline Integrity

Chemically bonded columns in good condition do not exhibit column bleed. If a high amount of column bleed is observed either the GC is in non optimum condition or the sample has been insufficiently cleaned up.

c. Adsorption/Degradation

In a poorly optimised GC system Endrin may be degraded to Endrin Aldehyde and Ketone by thermal isomerization. The presence of these degradation products indicates the GC is in non optimum condition.

3.8.2.2 Calibration

Internal standards (THE, PCB 112, PCB 198, and DCBP) are added to all samples, QC standards and calibration standards. The peak heights of these compounds are noted, these peak heights must be within a given range for each instrument before the GC is considered in optimum condition.

An Independent Standard consisting of all the target analytes in Iso-octane at 80% of the top calibration standard is GC analysed with each batch of samples. The concentration of each analyte is calculated using the calibration graphs for that batch. These results are plotted on Shewart control charts. These charts are prepared and maintained as described in NS 30 (1989). If the control chart indicates the analysis is out of control GC analysis of the batch is repeated.

In section 3.2.4.3 it was demonstrated that the response curve of the ECD was non linear. In order to minimise errors from extrapolation of the calibration curve the lowest calibration standard was used as the limit of quantification. The limit of detection for each anaytical procedure varied according to the size of the original sample, however, peaks smaller than the lowest calibration standard were not quantified.

3.8.2.3 Sample Preparation

Three methods are used to control possible errors from the sample preparation.

1. Blanks: A method blank consisting of 1 litre of deionised water (Method A, B or C) or an empty extraction thimble (Method D or E) is analysed with each batch of samples. The blank result is used to confirm that the analytical procedure is not resulting in a contribution to the measured amount of a determinand. Chromatograms of method blanks are visually inspected, if contamination is present the batch may be rejected. The blank is quantified and the concentration of each determinand calculated. If an individual determinand is positively detected in both a sample and blank, the blank is subtracted from the sample if the sample is greater than double the blank. If the blank is greater than the sample the blank is used as the detection limit.

2. Surrogate Standards: PCB 53 and εHCH are used as surrogate recovery standards in every extract. Surrogate standards are added to the sample extract immediately after extraction. These recovery standards are not used to correct data, but to provide an indication of the performance of the clean-up procedure. PCB 53 and εHCH both have a relatively high volatility and are late elutes from florisil and Alumina adsorption columns. Loses at this stage of the analysis due to incorrect evaporation or excessive retention on the clean-up columns will be exaggerated for these compounds and hence very easily detected. The percentage recovery of these compounds is calculated for each sample, samples with a recovery less than 40% may be rejected.

3. Laboratory Reference Materials (LRMs): Extraction and Process Recovery Standards
LRMs are not used for aqueous based samples, as discussed earlier the preparation of spiked reference materials is straight forward for aqueous based matrices, therefore, these are used in preference to LRMs for these matrices.

An Extraction Recovery Standard consisting of 1L of deionised water spiked with the target analytes and taken through the complete sample preparation procedure including extraction, clean-up and GC analysis is analysed with every batch of aqueous samples. The % recovery of each analyte is calculated. This is similar to the procedure used to correct the determined concentrations for loses of analytes which may have occurred during sample preparation.
The % recovery of analytes are also plotted on a Shewart control chart prepared and maintained as described previously.

A Process recovery standard consisting of a hexane solution of the target analytes is taken through the procedure excluding the extraction process. This standard is used for solid matrices to correct for loses of analytes during the sample preparation. This standard is not used to correct data for aqueous samples. The % recovery of analytes are also plotted on Shewart control charts prepared and maintained as described previously.

Sediment and Biota LRMs prepared as described in section 3.6.4.1 were analysed with each batch of sediment and biota samples, these were also plotted on Shewart control charts prepared and maintained as described previously.
Results identified as out of control by any of the above QC standards may lead to the re-analyses including sample preparation of the sample.

3.9 Evaluation of the Performance of the Optimised Analytical Procedures.

Programmes to study and protect environmental resources depend on data of proven quality. Experimental studies have shown that analytical results are often subject to errors. Inaccuracies in analytical data limit or prevent valid interpretations of the information available and subsequent decisions can be affected (NS 30, 1989.) Having optimised the various stages of analysis it is essential that the performance of a total method be fully validated and quality control procedures established to verify the routine performance of the method before it is put into routine use. Errors in analytical measurements are frequently classified as Random and Systematic (Hunt & Wilson 1986). These errors give rise to imprecision and bias respectively. These errors have different sources, remedies and consequences for the interpretation of data (Analytical Methods Committee 1995). Random errors determine the precision of analysis. They give rise to positive and negative deviations of results and are caused by uncontrolled and uncontrollable random variations in the factors that may affect analytical results e.g. slight variations in volumes etc. Systematic errors are a displacement of the mean value of many determinations from the true value. Systematic errors are commonly referred to as bias. Two forms of bias are commonly encountered i) Persistent bias, this applies over a long period of time and affects all data. Large persistent bias is apparent from the analysis of CRMs and participation in interlaboratory tests. This form of bias is unacceptable and may only be corrected by re-evaluation of the analytical protocol. If the persistent bias is small in relation to the random error it is generally acceptable, this form of bias is often very difficult to recognise and may only be identified by long term participation in interlaboratory tests and/or analysis of CRMs. ii) Catastrophic bias, this is an adventitious form of bias introduced by the failure of the system (e.g. misrecorded weight). This form of bias is normally large and readily identified by internal quality control.

Accuracy is the combination of bias and precision that determines how close an analytical measurement is to the true value. Determining the accuracy of an analytical method is essential before the method can be used to investigate environmental issues.

The estimation of precision is the first step in fully validating a method. Once the precision of a method has been established the bias can be fully investigated by analysis of certified reference materials and participation in interlaboratory tests.

3.9.1 Precision Tests

The primary aim of this study is to develop methods capable of detecting temporal and spatial changes in the concentrations of specific environmental concentrations of contaminants. In order to be able to do this it is essential that the precision of the analytical procedures be clearly defined. Analytical conditions are likely to vary from day to day and therefore the precision obtained in one batch of analysis is likely to be better than the precision obtained from many batches of analysis. For this reason, precision must be estimated from analyses taken from separate batches over a period of time.

In order to determine the long-term precision of an analytical procedure it is essential to have a stable well characterised material to analyse. As discussed earlier the preparation of spiked materials for the analysis of aqueous based matrices (e.g. river waters and sewage effluents) is relatively straightforward, their provision for non aqueous matrices (e.g. sediment and biota) is more complex. requiring that these matrices be considered separately.

3.9.1.1 Precision Testing For Aqueous Samples

The precision of the analytical procedures was determined using the principles outlined in NS 30 (1989) and Hunt & Wilson (1986).

Multiple batches consisting of duplicate "samples" of a blank, a low standard, a high standard, a sample and a spiked sample were processed through the entire analytical protocol. The composition of these "samples" is given in Table 84. In addition to these "samples" calibration and extraction recovery standards were processed, the "sample" results were recovery and blank corrected as if they were real samples.

Table 84 Composition of Samples used for Precision Testing.

	Liquid-Liquid	SPE	Effluents
Blank	1 l Deionised Water	1 l Deionised Water	1 l Deionised Water
Low Standard	1l deionised water spiked with the analytes at 90% of the top calibration standard	1l deionised water spiked with the analytes at 90% of the top calibration standard	1l deionised water spiked with the analytes at 90% of the top calibration standard
High Standard	1l deionised water spiked with the analytes at 90% of the top calibration standard	1l deionised water spiked with the analytes at 90% of the top calibration standard	1l deionised water spiked with the analytes at 90% of the top calibration standard
Sample	1l river water collected from White Cart at B764 Road Bridge.	1l river water collected from White Cart at B764 Road Bridge.	1l of treated effluent collected from Philipshill STW.
Spiked Sample	1l river water collected from White Cart at B764 Road Bridge and spiked with the analytes at 50% of the top calibration standard)	1l river water collected from White Cart at B764 Road Bridge and spiked with the analytes at 50% of the top calibration standard)	1l of treated effluent collected from Philipshill STW and spiked with the analytes at 50% of the top calibration standard

Analysis of Variance (ANOVA) was conducted on the results of these "samples" . This identified the different sources of variation (Between and Within batch) and allowed estimation of the total

standard deviation of the analytical procedure. Two statistical parameters, the within and between batch mean squares were used to estimate the within and between batch variances respectively. The estimates of the between and within batch variance were compared using an F test to determine whether there is a statistically significant between batch source of error.
The estimates of the between and within batch variance were used to calculate an estimate of the total standard deviation. Equations for the above calculations are shown below.

$$M_0 = \Sigma s_i^2/m$$

$$M_1 = n s_{bm}^2$$

$$s_t = \sqrt{((M_1 + (n-1)M_0)/n)}$$

M_0 is the within batch Mean Square
s_i is the standard deviation of the ith batch
m is the number of batches
M_1 is the between batch Mean Square
n is the number of replicate analysis within a batch
s_{bm} is the standard deviation of the batch means
s_t is the estimate of the total standard deviation.

The results of the ANOVA for the River waters liquid-liquid extraction, River waters SPE, and sewage effluents procedures are listed in Tables 85 to 87.

The Association of Directors and Rivers Inspectors of Scotland (ADRIS) Chemists group have indicated that it is desirable for an analytical procedure to have precision < 20 % in order to be suitable for routine environmental monitoring. The three methods tested met this precision target for all of the analytes tested. For the high standard and the spiked sample the precision was <10 % for all the target analytes using the 3 methods. This confirms that the methods have fully met the requirement set by ADRIS.

However, the precision of all three methods decreases as the analyte concentration approaches the limit of quantification. Precisions of between 10 and 20 % were recorded for the majority of analytes in the low standard. It is widely accepted that the precision of an analytical method is poorer as the analyte concentration decreases (Horwitz et al., 1980; ACS Committee on Environmental Improvement 1980; Holden et al., 1983; Ramsey et al., 1992; Clark and Whitfield 1994; Horwitz and Albert 1995). The ADRIS chemists method performance criteria does not take this factor into account. It is suggested that more realistic performance targets may be obtained by the use of the Horwitz equation as described in section 3.9.1.2.

Table 85 Results of Precision Test Determination of OCPs, PCBs, SynPyr, by Solid Phase extraction.

	Low Standard					High Standard					Spiked River Water				
Compound	M_1	M_0	F_{Value}	S_T	$\%S_T$	M_1	M_0	F_{Value}	S_T	$\%S_T$	M_1	M_0	F_{Value}	S_T	$\%S_T$
HCBD	0.051	0.010	5.040	0.175	8.98	0.749	0.232	3.22	0.701	4.01	0.397	0.244	1.625	0.566	6.00
α HCH	0.084	0.048	1.727	0.258	13.53	0.392	0.376	1.041	0.619	3.177	0.564	0.543	1.037	0.744	8.44
HCB	0.405	0.086	4.738	0.495	10.11	1.127	0.863	1.305	0.997	2.013	0.665	0.176	3.782	0.648	2.798
β HCH	0.891	0.174	5.132	0.729	8.294	7.120	1.751	4.111	2.116	2.333	3.097	1.028	3.012	1.437	3.169
γ HCH	0.042	0.042	1.014	0.205	5.149	0.898	0.324	0.782	0.782	1.689	1.277	0.577	2.211	0.963	5.946
PCB 28	0.364	0.110	3.306	0.487	10.15	1.393	1.031	1.352	1.101	2.226	0.420	0.468	0.898	0.666	2.726
PCB 52	0.245	0.143	1.710	0.378	9.669	3.762	0.924	4.071	1.531	3.190	2.631	0.782	3.365	1.306	5.725
Aldrin	0.031	0.081	3.911	0.141	6.970	0.325	0.242	1.343	0.532	3.181	0.548	0.119	4.584	0.577	7.553
OP DDE	0.131	0.104	1.259	0.343	17.29	1.824	0.526	3.469	1.084	6.600	1.115	0.502	2.220	0.899	11.46
PCB 101	0.079	0.022	3.563	0.224	11.16	2.047	1.390	1.474	1.311	2.704	0.893	1.557	0.574	1.107	5.840
Endo A	0.427	0.239	1.790	0.577	16.71	3.710	1.472	2.521	1.610	4.672	4.972	1.946	2.555	1.860	10.54
Dieldrin	0.284	0.052	5.500	0.409	16.80	4.718	1.281	3.683	1.732	4.341	2.084	0.653	3.194	1.169	6.204
PP DDE	0.310	0.172	1.799	0.491	11.58	2.913	0.341	8.548	1.276	2.584	2.476	0.967	2.572	1.314	6.653
Endrin	0.466	0.245	1.899	0.596	12.03	3.381	2.871	1.177	1.768	3.901	2.016	1.042	1.936	1.236	6.926
Endo B	1.048	0.142	7.378	0.771	15.07	7.481	5.567	1.344	2.554	5.809	1.279	0.658	1.945	0.984	5.233
PCB 101	0.078	0.022	3.563	0.224	11.16	2.047	1.389	1.474	1.311	2.704	0.893	1.556	0.574	1.107	5.840
PP DDD	0.762	0.229	3.327	0.704	17.13	1.635	0.646	2.530	1.068	2.410	1.698	0.484	3.506	1.045	6.049
OP DDT	0.201	0.106	1.887	0.392	10.24	10.84	7.422	1.460	3.022	6.878	1.858	1.123	1.655	1.221	6.565
PCB 153	0.355	0.257	1.384	0.553	11.03	2.114	0.823	2.568	1.212	2.409	2.722	0.878	3.097	1.341	7.879
PCB 105	0.404	0.893	4.520	0.496	10.28	2.658	0.551	4.829	1.267	2.526	2.100	0.423	4.968	1.123	6.065
PP DDT	0.273	0.270	1.010	0.521	13.06	2.135	0.756	2.824	1.202	2.939	1.389	0.379	3.661	0.940	4.941
PCB 138	0.356	0.163	2.192	0.509	11.12	2.455	0.507	4.837	1.217	2.903	3.337	0.517	6.451	1.388	7.184
PCB 156	0.405	0.164	2.470	0.533	13.27	1.526	2.368	0.644	1.395	3.003	1.424	0.601	2.369	1.006	5.511
Bifentrhin	79.35	13.15	6.032	6.801	8.725	710.2	536.9	1.322	24.97	3.253	600.4	475.6	1.26	23.19	6.371
PCB 180	0.374	0.152	2.455	0.513	12.39	1.435	0.642	2.236	1.019	2.188	0.759	0.622	1.221	0.831	4.521
Cis Permethrin	2.342	1.509	1.552	1.388	1.868	757.2	790.7	0.958	27.82	3.722	61.61	63.33	0.973	7.904	2.152
Trans Permethri	45.72	31.61	1.446	6.218	8.296	1218	457.4	2.662	28.94	3.915	142.8	127.4	1.120	11.62	3.506
Mean					12.29					3.719					6.282

Table 86 Results of Precision Test for the Determination of OCPs, PCBs, SynPyr, by liquid-liquid extraction in River Waters.

	Low Standard					High Standard					Spiked River Water				
	M_1	M_0	F_{Value}	S_T	$\%S_T$	M_1	M_0	F_{Value}	S_T	$\%S_T$	M_1	M_0	F_{Value}	S_T	$\%S_T$
HCBD	0.519	0.138	3.761	0.573	19.22	4.026	1.348	2.986	1.639	4.098	1.036	0.514	1.451	0.880	9.0
HCB	0.357	0.163	2.191	0.510	10.4	6.237	1.453	4.293	1.961	4.902	1.706	0.318	2.086	1.006	4.7
γ HCH	0.215	0.049	4.398	0.363	8.3	4.205	1.365	3.080	1.669	4.172	1.097	0.649	1.691	0.934	3.7
PCB 52	0.381	0.098	3.868	0.489	11.6	3.362	1.248	2.695	1.518	3.795	2.106	0.868	2.425	1.220	5.1
Aldrin	0.095	0.037	2.595	0.256	7.0	3.427	1.156	2.964	1.514	3.784	1.037	0.625	1.659	0.912	9.9
PCB 101	0.903	0.326	2.771	0.784	17.3	5.126	1.357	3.777	1.800	4.501	2.067	1.730	1.195	1.378	6.4
Dieldrin	1.036	0.327	3.172	0.826	19.2	4.964	1.963	2.529	1.861	4.653	1.389	1.490	0.932	1.200	4.8
Endrin	1.756	0.524	3.354	1.068	17.87	3.857	1.547	2.493	1.644	4.110	2.134	2.754	0.775	1.563	7.2
PP DDT	1.036	0.965	1074	1.00	18.99	12.63	8.070	1.565	3.217	8.043	3.573	1.671	2.138	1.619	6.9
PCB 180	0.803	0.207	3.876	0.711	14.9	3.287	1.390	2.365	1.529	3.823	2.496	1.893	1.319	1.481	6.4
Cis Permethrin	9.357	11.73	0.798	3.247	4.359	177.2	77.60	2.284	11.29	1.4	178.3	87.6	2.035	11.53	3.4
Trans Permethri	10.36	12.36	0.839	3.371	4.581	182.4	79.00	2.308	11.43	1.4	138.6	72.5	1.912	10.27	2.9
Mean					12.81				3.423						5.87

Table 87 Results of Precision Test for the Determination of OCPs, PCBs, SynPyr, by liquid-liquid extraction in Sewage Effluents.

	Low Standard					High Standard					Spiked Sewage Effluent				
	M_1	M_0	F_{Value}	S_T	$\%S_T$	M_1	M_0	F_{Value}	S_T	$\%S_T$	M_1	M_0	F_{Value}	S_T	$\%S_T$
HCBD	0.363	0.487	0.745	0.652	19.9	12.36	3.370	3.667	2.804	7.0	2.108	0.514	4.101	1.145	13.8
HCB	1.080	0.383	2.820	0.855	18.5	8.264	3.156	2.618	2.390	6.0	3.026	0.318	9.518	1.293	6.6
PCB52	0.381	0.137	2.778	0.509	12.9	5.367	4.267	1.258	2.195	5.5	1.892	0.868	2.179	1.175	5.5
Aldrin	0.147	0.259	0.569	0.450	15.6	6.267	4.367	1.435	2.306	5.8	0.850	0.425	2.000	0.798	9.8
PCB 101	1.007	0.368	2.736	0.829	19.9	7.896	5.167	1.528	2.556	6.4	3.158	1.730	1.825	1.563	7.7
PCB 180	1.258	0.358	3.514	0.899	19.2	8.965	4.309	2.081	2.576	6.4	3.067	1.893	1.620	1.575	7.7
γ HCH	1.274	0.347	3.671	0.900	18.2	4.390	3.247	1.352	1.954	4.9	1.257	0.649	1.937	0.976	4.8
Dieldrin	1.170	0.470	2.489	0.906	19.0	5.671	6.258	0.906	2.442	6.1	2.570	1.490	1.725	1.425	6.8
Endrin	1.081	0.596	1.814	0.916	19.7	7.236	3.108	2.328	2.274	5.7	3.270	2.754	1.187	1.736	8.5
PP DDT	1.636	0.485	3.373	1.030	19.6	13.26	9.029	1.468	3.338	8.3	4.158	1.671	2.488	1.707	8.4
Cis Permethrin	89.17	24.36	3.661	7.534	10.7	177.2	54.89	3.214	10.77	1.35	142.4	55.87	2.548	9.956	3.3
Trans Permethri	97.36	36.89	2.639	8.193	11.8	184.3	71.00	2.597	11.30	1.4	168.8	44.26	3.814	10.32	3.2
Mean					17.08					5.4					7.18

3.9.1.2 Precision testing for Solid Matrices

As indicated earlier solid matrices cannot be easily spiked to provide low and high standards. Therefore precision testing was carried out by analysing multiple batches of duplicate "samples" of the LRMs discussed in section3.6.4.1. In addition to these "samples" calibration and process recovery standards were processed with the samples, the "sample" results were recovery and blank corrected as if they were real samples. These results were statistically analysed as described previously.

The results of the ANOVA are presented in Table 88 and 89.

Table 88 Results of Precision Test on Ythan Mussels ng/g

Compound	Mean	M1	MO	F	St	%
PCB 28	2.9	0.801	0.215	3.724	0.713	24.6
PCB 52	21.8	3.137	0.743	4.224	1.393	6.4
PCB 101	78.9	7.607	2.036	3.736	2.196	2.8
PP DDE	5.1	0.947	0.418	2.266	0.826	16.2
PCB 118	64.3	7.138	3.218	2.218	2.276	3.5
PCB 153	63.8	6.358	4.128	1.540	2.289	3.6
PCB 138	92	9.25	4.021	2.300	2.576	2.8
PCB 180	6.3	0.898	0.509	1.764	0.839	13.3
DIELDRIN	3.3	0.726	0.427	1.700	0.759	23.0
PP DDD	7.4	0.829	0.416	1.993	0.790	10.7
PP DDT	2.2	0.481	0.106	4.538	0.542	24.6

Table 89 Precision test on Clyde Estuary Sediment ng/g

Compound	Mean	M1	MO	F	St	%
PCB 28	17.3	2.039	1.842	1.107	1.393	8.1
PCB 52	20	4.180	2.560	1.633	1.836	9.2
PCB101	9.2	1.587	1.482	1.071	1.239	13.5
PP DDE	7.5	0.957	0.857	1.117	0.952	12.7
PCB 118	10.6	1.260	0.965	1.305	1.055	10.0
PCB153	11.5	1.278	1.108	1.153	1.092	9.5
PCB 138	10.9	2.030	1.028	1.975	1.237	11.3
PCB 180	6.6	0.587	0.329	1.786	0.677	10.3
DIELDRIN	7.3	0.839	0.411	2.042	0.791	10.8
PP DDD	8.6	0.803	0.278	2.888	0.735	8.5
PP DDT	2.4	0.402	0.207	1.942	0.552	23.0

At present there are no clear guidelines from International Monitoring Organisations (e.g. ICES, JMP etc.) on the minimum performance required for the analysis of biota and sediments for persistent organics (Quasimeme 1995).

In the absence of clear guidelines from European monitoring programs prior to 1993 Quasimeme (Quality Assurance of Information for Marine Environmental Monitoring in Europe) set the following minimum criteria for target bias and precision. Quasimeme is a collaborative project between European marine institutes to improve the quality of data submitted to NSTF and JMP (OSPARCOM) marine monitoring programmes.

An analytical laboratory should be able to distinguish between two samples which differ by 50 % in concentration with 95% confidence.

The Horwitz equation (e.g. Horwitz 1995; Clark 1996; Horwitz 1980) has become an accepted method of estimating between and within laboratory variance of an analytical method irrespective of the analyte or matrix. The Horwitz equation is given below

$$RSD_b = 2^{(1-0.5\log C)} \cong 2C^{(-0.1505)}$$

Where: RSD_b is the between laboratory RSD.

C is the concentration of analyte on a mass/mass basis.

log is the logarithm to the base 10.

Using this equation the between laboratory RSD is estimated as 25 and 45 % at 50 and 1 ng/g concentrations repectively. A range of interlaboratory exercises have demonstrated that the within laboratory RSD is between a half and two thirds of the between laboratory RSD (Horwitz 1995). Therefore, the measured RSD should lie within 12.5 -19 % at the 50 ng/g level and between 22.5 - 34% at the 1 ng/g level. The sediment and biota methods both achieved within laboratory RSDs of between 3 - 10 % at the 50 ng/g range, and RSDs of between 20-25 % in the 1 ng/g. The precision achieved by both the sediment and biota methods are better than predicted by the Horwitz equation.

3.9.2 Bias

3.9.2.1 Methods For Aqueous Samples

As indicated earlier bias can be checked by analysis of certified reference material or by participation in interlaboratory studies. At present the author is not aware of any aqueous certified reference materials for the target analytes, therefore participation in interlaboratory schemes is at present the only practicable method of checking bias of the aqueous analytical methods. Two schemes were participated in to determine the bias of these methods.

Aquacheck is an inter-laboratory check sample service. Approximately 8 samples per annum consisting of ground water and spiking standards are distributed to the participating laboratories. Using the supplied spike participating laboratories spike the groundwater and determine the concentration of the target analytes in the groundwater using their routine methods. The analytes and the concentration range covered by the scheme is summarised below.

Compound	Range	Compound	Range
Endrin	0-200ng/l	PCB 28	0-200 ng/l
Dieldrin	0-200 ng/l	PCB 52	0-200 ng/l
Aldrin	0-200 ng/l	PCB 101	0-200 ng/l
pp DDT	0-200 ng/l	PCB 118	0-200 ng/l
Lindane	0-200 ng/l	PCB 138	0-200 ng/l
Endosulphan	0-200 ng/l	PCB 153	0-200 ng/l
Hxachlorobenzene	0-50 ng/l	PCB 180	0-200 ng/l

Throughout the period of the project CRPB has participated in Aquacheck for the above determinands, this work was carried out by the author and CRPB analysts working under the authors supervision. The mean percentage bias recorded in CRPB's Aquacheck returns during this period is less than 5%.

The above scheme does not cover the complete range of target analytes. In particular no synthetic pyrethroids were covered by this scheme. Despite extensive investigation the author was unable to find a scheme which covered these compounds.

Following the presentation of an oral paper on the monitoring of pyrethroids in the environment CRPB were invited to participate as an "expert" laboratory in a Community Bureau of Reference (BCR) program to produce water reference materials certified for several families of pesticides.

This program identified the following compounds as representative of families of pesticides which are currently poorly represented in CRMs; Permethrin, Atrazine, Simazine, Fenitrothion, Fenamiphos, Parathion-ethyl, Carbryl, Linuron and Propanil. Certified reference materials are required to be well characterised homogenous stable materials. Organophosphorus pesticides, carbamate insecticides and organonitrogen herbicides have all shown instability in aqueous samples (Liska *et al.*,1992, Munch & Frebis 1993, Senseman *et al.*, 1992; Chiron *et al.*, 1993). In order to overcome this problem, this program focused on the preparation and certification of a freeze dried water sample.

Previous studies undertaken by BCR had indicated that the target pesticides were stable in freeze-dried water samples, following on from this study BCR invited 14 "expert" European environmental analytical laboratories to determine the concentrations of the target pesticides in samples of freeze dried water with a view to producing a certified reference material. All the participants in this exercise were required to follow a strict protocol. This involved 5 independent determinations of the concentrations of the pesticides in the freeze dried water, 5 independent determinations of a freeze dried blank water sample, 5 independent procedural reagent blanks, at least triplicate estimation of extraction efficiency and recovery, and the determination for each compound the range of the detectors linear response. For the majority of the target analytes the results of this study were very encouraging with reasonable agreement observed between the "expert" laboratories. Unfortunately, only two of the fourteen "expert" laboratories returned any data for permethrin. Of these laboratories the CRPB laboratory was the only laboratory to return a complete data set for permethrin. This poor response for permethrin reflects the extreme challenge that the determination of trace amounts of permethrin represents. It is difficult to draw conclusions from such an extremely small data set. The results of this initial interlaboratory test have been fully reported elsewhere (Barcelo *et* al., 1994). Further work is currently underway on the certification of the freeze dried water samples, the final stage of the interlaboratory test is expected to be complete by the end of 1996 and the freeze dried material available in 1997.

3.9.2.2 Methods For Solid Samples.

A wide range of certified reference material are available for the determination of PCBs in environmental samples. A small amount of BCR CRM No. 349 Cod-liver oil was available for use as a reference material. Duplicate 100mg portions of this reference material were analysed when conducting the biota precision test described earlier. The mean determined concentrations along with the certified values and tolerances are listed in Table 90.

Table 90 Mean Determined Concentrations in Cod-Liver Oil

Compound	Certified Value expressed as ug/kg	Uncertainty expressed as ug/kg	Determined Value as ug/kg
PCB 28	68	±7	63
PCB 52	149	±20	161
PCB 101	370	±17	363
PCB 118	454	±31	441
PCB 138 *	765	±45	742
PCB 153	938	±40	928
PCB 180	280	±22	275

* It has subsequently been shown that this value is the combined concentration of PCB 133 and 163.

The determined value is within the 95% confidence limits for each of the analytes.

Analysis of the CRM 349 only indicated bias for a very narrow range of analytes in cod oil. Bias in the determination of PCBs, α HCH, γ HCH, PP DDD, PP DDT, dieldrin, and Trans Nonachlor in sediments and biota was indicated by participation in the Quasimeme interlaboratory program. Throughout the period of this project the laboratory participated fully in the Quasimeme scheme for the measurement of PCBs and OCPs in marine sediments and biota using the above optimised methods. The results of these interlaboratory schemes have been fully published in Quasimeme Laboratory Performance studies Rounds 1-5 and in Wells & de Boer (1994). Results for the most recent exercise are summarised in Table 91.

No interlaboratory exercise for the determination of pyrethroid insecticides could be found to participate in.

Table 91 Recent Performance in Quasimeme Interlaboratory Exercise.

	QOR023SS & QOR024SS Standard Solution.			QOR022BT Cod Liver Oil			QOR026M Baltic Marine Sediment			QOR027MS Haringvliet Freshwater Sediment.		
Compound	Assigned Value µg/kg	Determined Conc µg/kg	% Error	Assigned Value ug/kg	Determined Conc µg/kg	% Error	Assigned Value ug/kg	Determined Conc µg/kg	% Error	Assigned Value ug/kg	Determined Conc µg/kg	% Error
PCB 28	458	479.92	4.8	8.8	<25	ND	0.71	1.16	63.4	36.1	33.6	-6.9
PCB 52	394	385.42	-2.2	36.8	38.5	4.6	1.37	1.41	2.9	31.9	30.93	-3.0
PCB 101	652	617.42	-5.3	59.2	59.7	0.8	3.27	3.21	-1.8	33.8	32.12	-5.0
PCB 105	415	438.56	5.7	17.5	9.5	-45.7	0.78	0.74	-5.1	6.4	8.24	28.8
PCB118	405	418.86	3.4	72.4	77.25	6.7	2.34	2.04	-12.8	24.6	25.03	1.7
PCB138	551	518.66	-5.9	248	211.7	-14.6	5.03	4.52	-10.1	33	28.39	-14.0
PCB153	583	570.08	-2.2	345	323.9	-6.1	5.74	5.21	-9.2	45.1	34.27	-24.0
PCB156	301	305.8	1.6	14.6	10.4	-28.8	0.48	0.42	-12.5	2.8	2.66	-5.0
PCB180	913	872.5	-4.4	106	99	-6.6	3.71	2.98	-19.7	25.4	20.4	-19.7
HCB	138	129.15	-6.4	79.1	111.6	41.1	0.55	0.55	0.0	13.1	15.24	16.3
pp DDE	520	527.91	1.5	760	583	-23.3	1.07	1.05	-1.9	7.6	7.01	-7.8
α HCH	152	135.68	-10.7	39.7	60.8	53.1	0.04	<0.1	ND	0.5	0.23	-54.0
γ HCH	158	153.45	-2.9	8	8.89	11.1	0.14	0.11	-21.4	0.6	0.36	-40.0
PP DDD	539	479.72	-11.0	94.8	90.6	-4.4	0.97	1.05	8.2	3.5	2.52	-28.0
PP DDT	795	869.63	9.4	68.3	71.8	5.1	0.49	<1.0	ND	1.7	<2	ND
Dieldrin	511	544.98	6.6	86.3	116.9	35.5	0.59	0.48	-18.6	1.2	0.41	-65.8
Trans Nonachlor	399	379.58	-4.9	361	423.9	17.4	0.05	<0.1	ND	0.8	<1.5	ND

Chapter 4

Mothproofing Chemicals in the Annick Water.

4.1 Introduction

Wool is a natural fibre with excellent textile properties which has led to its widespread use in the textile and carpet industries. When compared to man man-made fibres it has one serious limitation, its susceptibility to insect damage (Friedman et al., 1979). The larvae of a number of beetles, and moths (e.g. *Oecophoridae*, House Moths and *Tineidae*, Clothes Moths) feed on wool protein (keratin) and can cause severe damage to wool based products. To avoid damage and minimise this disadvantage, wool and wool blend fibres are chemically treated to prevent damage by insects. This treatment is known as mothproofing.

Since the late fifties a variety of synthetic organochlorine compounds have been used in the textile and wool processing industry as cheap and effective mothproofing agents (Duffield,1977). One of the first agents used was dieldrin but because of its high toxicity to aquatic life it was replaced by alternative formulations. The industry switched first to polychloro chloromethyl sulphonamido diphenyl ethers (PCSDs) and then to Flucofuron and Sulcofuron. Since the late 1980's these compounds have been largely replaced by the synthetic pyrethroid permethrin (Zabel et al., 1988). In the west of Scotland dieldrin was used as the principal mothproofing agent until 1978, Eulan was used between 1978 and 1983. Since 1983 permethrin has been used as the most important mothproofing agent (Redshaw CRPB, pers comm.). This investigation focuses principally on permethrin.

Extensive research has been published on the agricultural use of permethrin (Demoute, 1989) but this author is unaware of published data on the fate of permethrin derived from its use in the textile industry. In this study an attempt is made to track and determine the fate in the receiving water course of organochlorine compounds in the effluent of a sewage treatment works (STW) receiving trade wastes from an industrial wool processing works. The sewage treatment works is situated in Stewarton, a small town in Ayrshire, and the final effluent is discharged into the Annick Water.

4.2 Location and Background

Stewarton is a small town of approximately 6000 inhabitants situated 18 miles SSW of Glasgow and 5 miles NW of Kilmarnock. The town has a proud tradition of textile and clothing manufacture dating back to the mid 16th century. In the early 19th Century 18 mills were located in Stewarton, but to-day only one woollen mill is still in production and is located on the banks of the Annick Water in the centre of Stewarton. The effluent from this factory is discharged to the local sewerage system which transports it to Stewarton STW where it receives primary and secondary treatment before being discharged to the Annick Water. The measured dry weather flow of the STW is 0.028

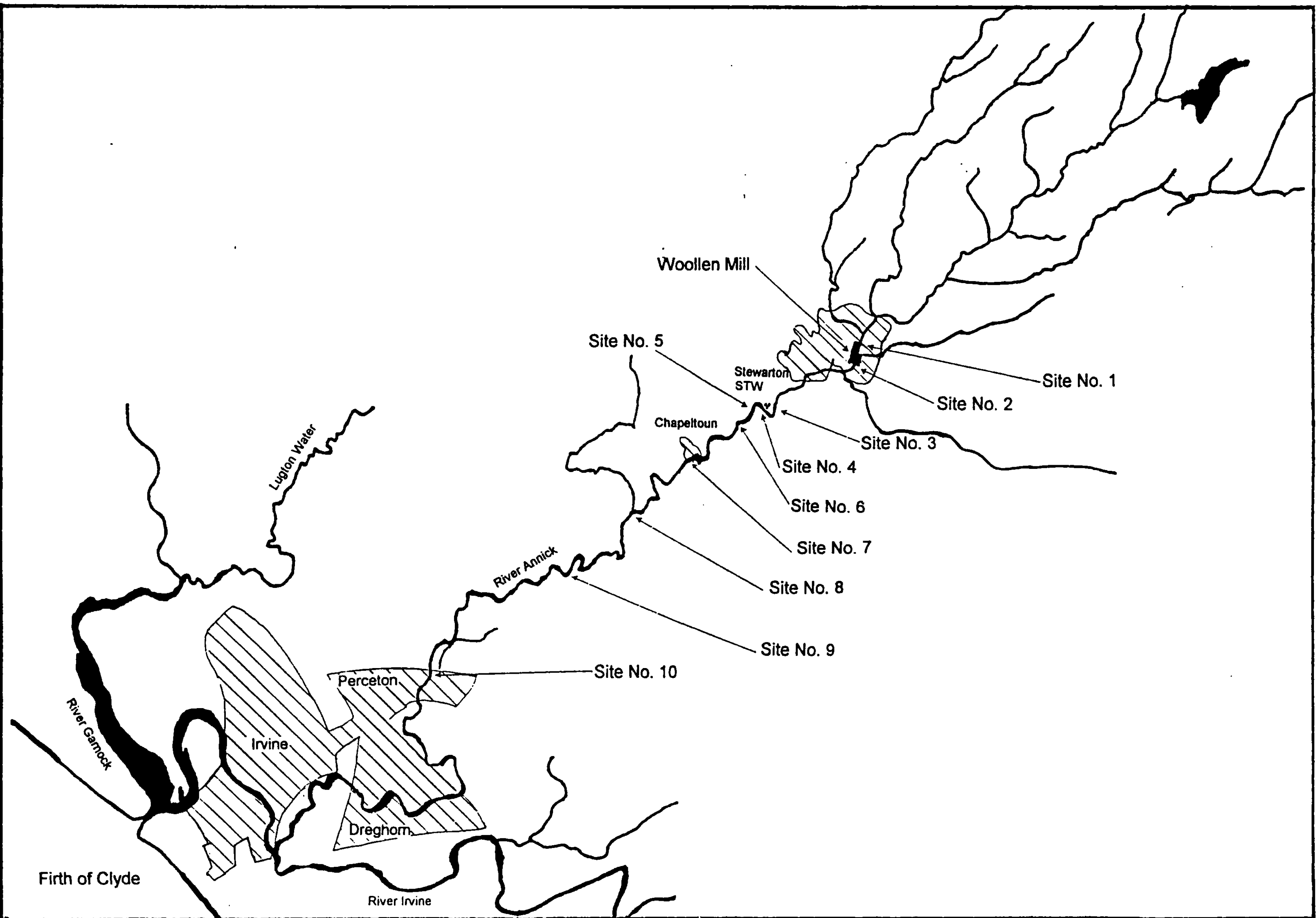
cumecs (C.R.P.B., Consent 1994), and the maximum flow permitted in the consent for the STW is 0.084 cumecs.

The Annick Water is a tributary of the River Irvine. The Annick Water rises to the east of the Long Loch in high marshy ground on the Renfrew / Ayrshire border, and flows down through Stewarton, Chapeltoun, Perceton and Dreghorn to reach its confluence with the River Irvine on the outskirts of Irvine 30.5 km from its source. The Annick Water catchment is shown graphically in Figure 11. The catchment area of the Annick Water is approximately 100 Km^2 and consists mainly of farmland. The average flow of the Annick is 3.3 cumecs with extremes of 0.041 cumecs (min) and 45.6 cumecs (max). The 95 % exceedence flow is 0.164 cumecs (C.R.P.B., 1985).

The chemical classification scheme adopted for rivers in Scotland classifies the complete length of the Annick Water as Class 1. This is the highest quality chemical class indicating that the water is clear, normally has a biochemical oxygen demand of less than 3mg/l, is well oxygenated and does not receive significant polluting discharges (Scottish Office, 1992). The biological classification scheme adopted for rivers in Scotland classifies the Annick Water in Stewarton as poor and the complete length of the Annick Water below Stewarton as very poor (Scottish Office, 1992).

In this study contamination by mothproofing chemicals is investigated as the possible explanation for the discrepancy between the chemical and biological classifications of the Annick Water.

Figure 11. The Annick Water Catchment.



4.3 Sampling Strategy

4.3.1 Effluent and River Waters

The inputs of mothproofing chemicals to the Annick Water were monitored monthly from January 92 to December 95 by determining the concentration of permethrin and organochlorine compounds in 24 hour composite samples of the effluent from Stewarton STW. Additionally occasional receiving water snap samples up and down stream of the STW discharge were collected. British National Grid Reference points for the sampling locations are given in Table 92, and are shown graphically in Figure 11.

Table 92. Location of effluent and river sampling sites.

Site	Grid Reference
Stewarton STW discharge to the Annick Water	NS 4075 4485
Annick Water upstream Stewarton STW	NS 4222 4546
Annick Water down stream Stewarton STW	NS 3945 4425

To save costs the industry combines the process of mothproofing with the dyeing of the wool. The mothproofing chemicals are added to the dyebath, and batches of wool are dyed and mothproofed simultaneously (Shepley <u>et al</u> 1980, Shaw 1994(b)). Spillages and run off from the transport of wool to and from dyebaths results in small amounts of mothproofing chemicals being constantly released to the sewerage system. The major inputs of mothproofing chemicals are released into the sewerage system as short episodic events when the dyebaths are emptied. This can result in high concentrations of mothproofing chemicals being discharged by the STW for short periods of time. Composite samples over 24 hours integrate these extreme concentrations and may produce low estimates of the maximum concentrations being discharged by the STW. To investigate the possibility of episodic high level releases, 12 separate 2 hour composite samples of the effluent from Stewarton STW were monitored in Oct. 93 and Nov. 93.

4.3.2 Sediments

Sediment is an important habitat for many organisms. Sediment may also act as a contaminant reservoir, integrating episodic inputs of pollutants over long time periods providing a forensic record of past and present contamination. In order to be adsorbed by the wool fibres mothproofing chemicals are highly lipophilic. It was anticipated that this lipophilic property would lead to rapid adsorption of these agents by suspended solids in the river environment . Sediment not the receiving water should be the ideal matrix to monitor the fate and transport of lipophilic contaminants. Sediments were sampled at 10 sites along the length of the Annick . A survey of sediments from the River Annick was carried out on 20/08/93.

Figure 12 Wool Processing Factory on the Banks of the Annick Water, Stwearton

Figure 13 Effluent Discharging from Stewarton STW to the Annick Water

Figure 14 Sediment Collection Immediately Downstream of Discharge from Stewarton STW

Figure 15 Sediment Collection at Site No.6 Down Stream of Stewarton STW

Figure 16 Sediment Collection at Site No 7 Chapeltoun.

Figure 17 Site No 8 Langlands Farm.

Figure 18 Site No. 9 Cunninghamhead

Figure 19 Site No. 10 Perceton

British National Grid Reference points of sediment sampling locations are given in Table 92 and are shown graphically in Figures 12-19.

Table 92 Sediment Sampling Locations on the Annick Water.

Site Number	Sample Description	Grid Reference
Site No. 1	Up Stream Wool processing factory	NS 423 461
Site No. 2	Downstream Wool processing factory	NS 422 460
Site No. 3	Up Stream Stewarton STW	NS 409 449
Site No. 4	Stewarton STW Outlet Pipe	NS 408 448
Site No. 5	100M Downstream Stewarton STW	NS 407 449
Site No. 6	500M Downstream Stewarton STW	NS 405 449
Site No. 7	Chapeltoun	NS 394 442
Site No. 8	Langlands Farm	NS 385 432
Site No. 9	Cunningham Head	NS 375 424
Site No. 10	Perceton	NS 350 406

4.3.3 Biota

As previously stated mothproofing chemicals are highly lipophilic and thus also likely to accumulate in food chains. In contrast with marine biota studies there is relatively little published information on the occurrence of pesticides and mothproofing chemicals in a range of freshwater fish. The only fish which has been studied extensively is the eel (*Anguilla anguilla*).

The eel is a sea fish which spends a large part of its life in fresh or brackish waters. The life history of the eel is remarkable, spawning in the Sargasso Sea the resultant larvae are carried by the Gulfstream over a 3 year period to the European coastline, where they metamorphose into elvers which migrate into the rivers and streams of Northern Europe. Eels remain in rivers or streams for 4 -10 years, feeding on smaller fish, crayfish, frogs, mussels, snails and the larvae of insects. During this period they are known as yellow eels. The growth rate of eels depends on their food and river temperature. Eels typically reach a length of 8 cm in their first year in a river or stream and 17 - 19 cm length during their second year. Mature eels typically grow to 50-80 cm length. After spending 4 - 10 years in the freshwater environment mature eels change their appearance. The eyes increase in size, the muscles of the jaws shrink, the head becomes pointed and the skin becomes dark on the back, and silvery on the belly. These mature eels are known as silver eels. Gradually the silver eel ceases feeding and in September - October migrates back to the sea. The details of the final stage of the eels life are still unclear, however, the eels eventually travel back to the Sargasso Sea where they spawn.

In the freshwater stage of its life cycle the eel because of its high lipid content is an excellent biomonitor for pesticides and other lipophilic contaminants. The eel is widely distributed throughout Europe, it is non-migratory during this period living in localised areas of river systems, reasonably tolerant to pollution, has a high fat content which does not undergo seasonal changes due to sexual maturation, and is popular for human consumption.

Eels are abundant throughout west of Scotland watercourses (Doughty pers comm.). These characteristics together with its local availability suggested that the eel would be a useful biomonitor for the Annick Water.

Previous workers (de Boer and Hagel 1994) have recommended sampling yellow eels in early spring. The rationale behind this is that all silver eels from the previous season will have left the river the previous Autumn and remaining mature eels will not yet have begun the transformation to silver eels. This strategy ensures that all eels sampled are at a similar and appropriate life stage.

Due to operational difficulties it was not possible to sample for eels in the Annick during this favoured spring period. In this study eels were sampled during Autumn. During this season the transformation of mature eels from yellow to silver is complete, and the two growth stages can be easily distinguished. In low temperatures such as those frequently encountered in early spring the eel is passive and may spend much of its time hidden in mud or below stones in frost free places. Contrastingly in Autumn the yellow eel is active having spent the summer feeding. It is the authors view that Autumn sampling of eels offers the advantage that the yellow eels are active, easily caught, and have spent the previous months feeding and thus they more accurately reflect current river conditions.

In an effort to ensure that the eels analysed were of a similar age and hence of similar exposure period to the mothproofing chemicals, only eels within the size range 25 - 40 cm were selected for analysis. It was hoped that these eels would be of between 3-4 years age. Eels were sampled by electric fishing. This sampling was carried out on 31/8/94 and 22/9/94 by CRPB Biology staff as part of a survey of fish populations in the Annick Water. A sampling strategy to sample eels from the same locations as were sampled for sediments was planned. However, due to size and weight of the electric fishing equipment not all of the sediment sampling sites could be accessed. The sites used for electric fishing are listed in Table 93.

Table 93 Electric Fishing Locations

Site No.	Site Description	Grid Reference
1	Annick Water upstream Wool processing factory	NS 452483
2	Annick Water down stream Wool processing factory	NS 422455
3	Annick Water upstream Stewarton STW	NS 411452
4	Annick Water Chapeltoun	NS 393440
5	Annick Water Langlands Farm	NS 385432
6	Annick Water Cunningham Head	NS 375424
7	Annick Water Annick Lodge	NS 361418
8	Glazert Burn upstream of Langlands farm	NS 385433

4.4 Experimental Methods

4.4.1 Effluent and River Water Samples

All of the effluent samples were collected using an EPIC model 1011T autosampler. All tubing or parts that came into contact with the sample were either of glass or PTFE.

River water samples were collected from bridges using a 1l stainless steel sampling can which was filled from mid channel in the water course. Degradation of the analytes was minimised by the addition of 50 mls of hexane to the effluent and river water samples immediately upon receipt of the samples in the laboratory. All effluent and river samples were processed using the analytical and quality control procedures outlined in section 3.7.

4.4.2 Sediments

The Annick Water is a narrow fast flowing river with few quiescent stretches. This made the collection of sediment samples difficult. Surface sediments were collected by wading into the river and carefully scooping the surface layer of sediment with a (50ml) glass jar. The glass jar was sealed with an Aluminium foil lined top. The samples were transported back to the laboratory where they were frozen to -20 °C and freeze dried the following day using an Edward's K4 Modulyo freeze dryer, set at -70 °C.

Figures 12 to 19 show the Annick Water sediment collection sites.

The particle size distribution of the freeze dried sediments were determined by size fractionation using a series (2, 1, 0.5 mm) of brass sieves. The greater than 2 mm fraction was discarded and the remaining fractions were combined and used for all the subsequent determinations.

Total organic carbon was measured using a Dohrman DC-190 TOC analyser fitted with a Dohrman 183 boat analyser. Approximately 2g of the freeze dried sediment was ground using a mortar and pestle and a 10 mg aliquot of this was used for TOC determinations. The instrument was calibrated using potassium hydrogen phthalate, and the accuracy of the determination checked by analysing the certified reference material MESS-1 with each batch of sediments.

Organochlorine compounds were determined on the less than 2mm fraction of the freeze dried sediments using the analytical and quality control procedures outlined in section 3.7.

4.4.3 Biota

Eels were collected by CRPB Biology staff using Millstream FB3F pulsed DC (100pps) electric fishing gear powered by a 2kVA alternator. A single anode and cathode were employed without stop nets. The area fished ranged from 154m^2 upstream of the wool processing factory to 651 m^2 at Cunningham Head. The species and number of fish caught at each location were recorded. Eels were caught at all locations except Chapeltoun. At all sites where eels were caught five eels in the

size range 25 - 40 cm were selected and placed in polythene bags for transport to the laboratory. On receipt in the laboratory the eels were deep frozen prior to sample preparation.

The frozen eels were allowed to partially thaw, the length and weight of each eel was measured and recorded. The eels were skinned and 15g samples of muscle fillets were taken from each of the eels. Muscle fillets from the eels captured at the same site were placed in 250ml glass jars which had been pre-washed with Hexane and Acetone. The combined muscle fillets were deep frozen and freeze dried as per the sediments. The freeze dried fillets were macerated using an Ultra Turrex homogeniser. 5 g portions of the homogenised muscle were taken for organochlorine and extractable material determination. The organochlorine and extractable material determination was carried out using the analytical procedures outlined in section 3.7.

4.5 Results

4.5.1 Effluent Samples

Between Jan 1993 and Dec. 1995, 36 twenty-four hour composite samples of the effluent from Stewarton STW were analysed for the 28 organochlorine compounds listed in Table 3.

19 of the selected compounds were never positively detected. These compounds included PCBs, the industrial solvents Hexachlorobutadiene, Hexachlorobenzene and the insecticides endosulfan, aldrin and endrin.

9 compounds were frequently detected in the effluent from Stewarton STW. These were α Hexachlorohexane, β Hexachlorohexane, γ Hexachlorohexane, para para DDE, para para DDD, para para DDT, dieldrin, cis and trans permethrin. The results of the analysis for the compounds which were frequently detected are summarised in Table 93.

Table 93 Compounds frequently detected in 24 Hour Composite Samples of Effluent from Stewarton STW 1993-1995.

Compound	No. of Observations	% of Results Compound was positively detected	Mean Concentration of Positive Results ng/l	Median Concentration of Positive Results ng/l	Maximum Concentration ng/l
cis permethrin	36	81	338	189	2140
trans permethrin	36	81	328	255	1660
dieldrin	36	75	6.1	6.2	10.1
pp DDE	36	14	6.6	5.1	19.3
pp DDD	36	6	2.1	2.1	2.7
pp DDT	36	19	24.4	10.6	102
α HCH	36	33	2.7	2.2	6.41
β HCH	36	53	10.1	6.6	36.9
γ HCH	36	95	54.7	37	216

Lindane (γ HCH) was present in 95 % of the samples and was the most frequently detected organochlorine compound. Effluent concentrations of Lindane ranged from <1 - 216 ng/l, with typical concentrations of 40 ng/l. The isomers of Lindane, α HCH and β HCH were detected less frequently and at much lower levels than Lindane. The concentrations detected of α and β HCH are close to the limit of detection of the analytical method and this is reflected in their lower frequency of detection.

Permethrin was frequently detected in the effluent (81% of samples). Permethrin was present in the effluent at much higher concentrations than Lindane. Effluent concentrations for total permethrin (total permethrin = sum of cis and trans permethrin) ranged from <20 to 3800 ng/l with typical total permethrin concentrations of 480ng/l The average ratio of cis to trans permethrin in the effluent was 48:52.

Dieldrin was detected in the majority (75%) of samples. The concentrations ranged from <1ng/l to 10.1 ng/l with typical concentrations of 6ng/l.

pp DDT and its metabolites pp DDD and pp DDE were detected infrequently in the effluent (19, 6 and 14 % of samples). pp DDT was the most frequently detected member of this group of compounds, concentrations ranged from <1 - 102 ng/l.

The concentrations of compounds positively detected in 2 hourly composite samples of effluent over a 24 hour period from Stewarton STW are listed in Tables 94 and 95. These results are similar to those obtained from the monitoring of the 24 hour composite samples. The 9 compounds detected in 24 hour composite samples were also detected in the majority of the 2 hour composite samples.

Table 94. 24 Hour Pattern of Concentration (ng/l) of Organochlorines in Stewarton STW Effluent October 93.

Sample Description Date Time	cis permethrin	trans permethrin	dieldrin	pp DDE	pp DDD	pp DDT	α HCH	β HCH	γ HCH
20/10/93 12:00	110	133	<4.0	<4.0	<4.0	<4.0	<2.0	19.1	11.8
20/10/93 14:00	93	112	4.1	<4.0	<4.0	<4.0	<2.0	19.5	12.1
20/10/93 16:00	99	126	4.6	<4.0	<4.0	<4.0	<2.0	29.3	13.1
20/10/93 18:00	77	107	4.3	<4.0	<4.0	<4.0	<2.0	16.4	11.8
20/10/93 20:00	246	313	9.6	<4.0	<4.0	7.9	<2.0	112.9	15.7
20/10/93 22:00	224	274	10.7	<4.0	<4.0	8.7	24.3	88.5	16.3
21/10/93 0:00	178	231	8.4	<4.0	<4.0	7.0	2.7	72.7	15.0
21/10/93 02:00	226	244	7.5	<4.0	<4.0	6.9	2.4	56.5	14.7
21/10/93 04:00	154	179	5.9	<4.0	<4.0	5.4	<2.0	43.8	12.9
21/10/93 06:00	233	201	6.8	<4.0	<4.0	5.7	<2.0	53.2	16.1
21/10/93 08:00	211	212	6.8	<4.0	<4.0	5.9	4.1	45.9	15.9
21/10/93 10:00	165	168	5.7	<4.0	<4.0	4.8	3.9	45.7	15.9
Mean result	168	192	6.5	<4.0	<4.0	5.7	4.3	50.29	14.3

Table 95. 24 Hour pattern of Concentrations (ng/l) of Organochlorines in Stewarton STW Effluent November 93.

Sample Description Date Time	cis permethrin	trans permethrin	dieldrin	pp DDE	pp DDD	pp DDT	α HCH	β HCH	γ HCH
23/11/93 10:00	624	804	<4.0	<4.0	<4.0	<4.0	<2.0	10.1	21.3
23/11/93 12:00	665	854	<4.0	<4.0	11.0	24.1	4.0	14.4	23.1
23/11/93 14:00	560	721	14.5	<4.0	10.1	24.4	<2.0	21.0	25.7
23/11/93 16:00	530	750	10.9	<4.0	<4.0	26.1	<2.0	13.9	24.9
23/11/93 18:00	479	675	11.5	<4.0	7.4	37.7	<2.0	20.3	23.6
23/11/93 20:00	579	731	12.7	<4.0	11.0	26.4	4.7	16.1	24.9
23/11/93 22:00	259	281	11.5	<4.0	<4.0	18.0	<2.0	19.8	24.1
24/11/93 00:00	458	595	10.2	<4.0	<4.0	<4.0	5.16	19.6	24.6
24/11/93 02:00	416	607	13.9	<4.0	9.0	<4.0	7.0	23.1	24.3
24/11/93 04:00	426	653	10.0	<4.0	8.5	<4.0	4.1	13.8	24.3
24/11/93 06:00	367	545	10.9	<4.0	8.7	<4.0	<2.0	13	22.1
24/11/93 08:00	355	496	11.0	<4.0	9.2	27.6	4.1	21.3	77.7
Mean result	488	656	10.4	<4.0	7.6	17.0	3.4	17.2	23.9

The major organochlorine contaminant detected in the effluent on both sampling occasions was permethrin. Permethrin was detected in all the effluent samples with the total permethrin concentration ranging from 184 - 1520 ng/l with a mean concentration of 751 ng/l. The average ratio of cis to trans permethrin in the effluent sample was 44:56.
Dieldrin was detected in the majority of samples at concentrations ranging from <4.0 - 14.5 ng/l with a mean concentration of 8.5 ng/l.

pp DDT was also detected in the majority of samples at concentration ranging from <4.0 - 37.7 ng/l with a mean concentration of 11.4 ng/l. The metabolites of DDT, pp DDE and pp DDD were detected in a few samples at concentrations <12 ng/l.

γ HCH was detected in all the effluent samples at concentrations from 11.8 - 77.7 ng/l with a mean concentration of 19.1 ng/l. α HCH was positively detected in approximately half the samples but at levels much lower than γ HCH with a range of <2.0 - 24.3 ng/l and a mean concentration of 3.9 ng/l. β HCH was detected more frequently and at higher concentrations on both of these sampling occasions than would be expected from the 24hour composite samples.

The overall pattern of contamination and concentrations of contaminants detected in both the 24 and 2 hour composite samples is similar. The range of concentrations of contaminants detected in the 2 and 24 hour composite samples is also similar for compounds excepting β HCH. This indicates that although mothproofing is carried out in batches at the factory the subsequent sewage treatment process evens out the discharge levels of mothproofing chemicals and further confirms that 24 hour composite samples are representative of typical concentrations discharging into the Annick Water.

4.5.2 River Water Samples

Over a 3 year period (1993-95) 36, river samples from sites up and down stream of the STW were analysed for the 28 organochlorine compounds listed in Table 3. Only α and γ HCH were positively detected in these samples. The results for α and γ HCH are summarised in Tables 96 and 97.

Table 96 Concentrations (ng/l) of Organochlorines detected in the Annick Water upstream of Stewarton STW Jan 1993- Dec1995.

Compound	No. of Observations	% of Positive Results	Mean Concentration (ng/l) of Positive Results	Median Concentration (ng/l) of Positive Results	Maximum Result (ng/l)
α HCH	36	1	2.3	2.3	2.3
γ HCH	36	3	4.1	3.4	6.8

Table 97 Concentrations (ng/l) of Organochlorines detected in the Annick Water down stream Stewarton STW 1993-1995

Compound	No. of Observations	% of Positive Results	Mean Concentration (ng/l) of Positive Results	Median Concentration (ng/l) of Positive Results	Maximum Concentration (ng/l)
α HCH	36	2	2.3	2.5	4.1
γ HCH	36	4	5.4	6.3	11.3

This suggests that analysing the receiving water is not an effective means of monitoring the release of organic contaminants from Stewarton STW.

4.5.3 Sediments

The results for total organic carbon and particle size distribution in the sediments are presented in Table 98 and shown visually in Figures 20 and 21.

Table 98. Percentage Total Organic Carbon and Particle Size Distribution as % of Total Weight in Sediments from the Annick Water

Description	TOC %	0-0.5mm	0.5-1mm	1-2mm	>2mm
upstream wool processing factory	1.02	59.29	27.12	8.06	5.53
downstream wool processing factory	0.50	7.81	21.70	27.72	42.77
upstream Stewarton STW	0.72	33.64	16.50	26.76	23.10
Stewarton STW outlet	1.30	20.85	20.57	17.19	41.39
100M downstream Stewarton STW	0.56	16.78	20.84	25.41	36.97
500M downstream Stewarton STW	1.04	76.64	15.01	4.93	3.42
Chapeltoun	0.62	7.72	16.79	27.36	48.12
Langlands Farm	0.42	9.65	24.10	27.78	38.48
Cunningham Head	0.39	18.20	42.50	31.21	8.09
Perceton	1.30	89.08	7.60	1.46	1.83

Figure 20: %TOC in Annick Water Sediments

Figure 20 % TOC in Annick Water Sediments.

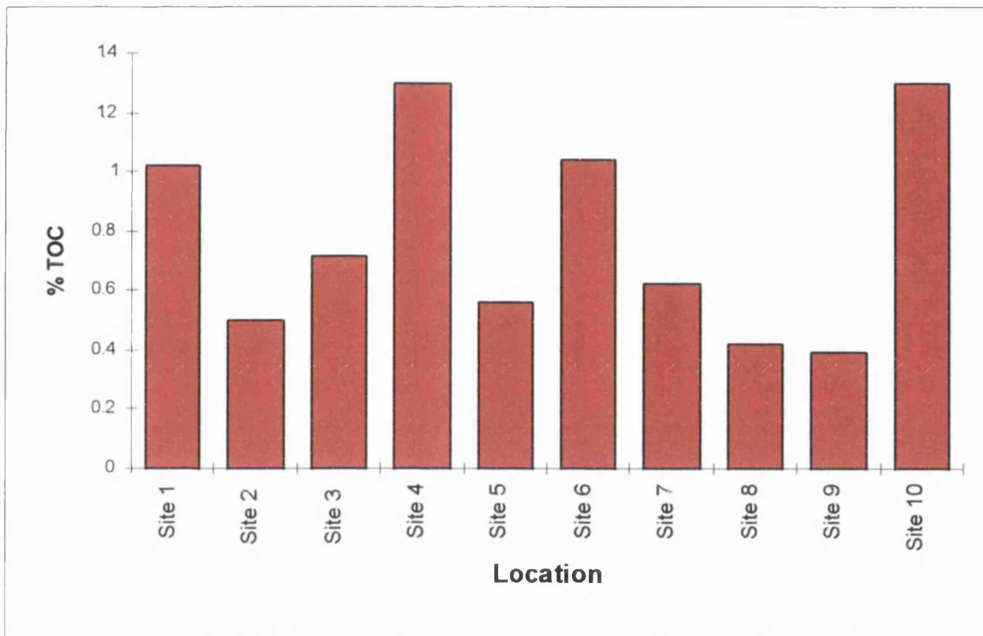
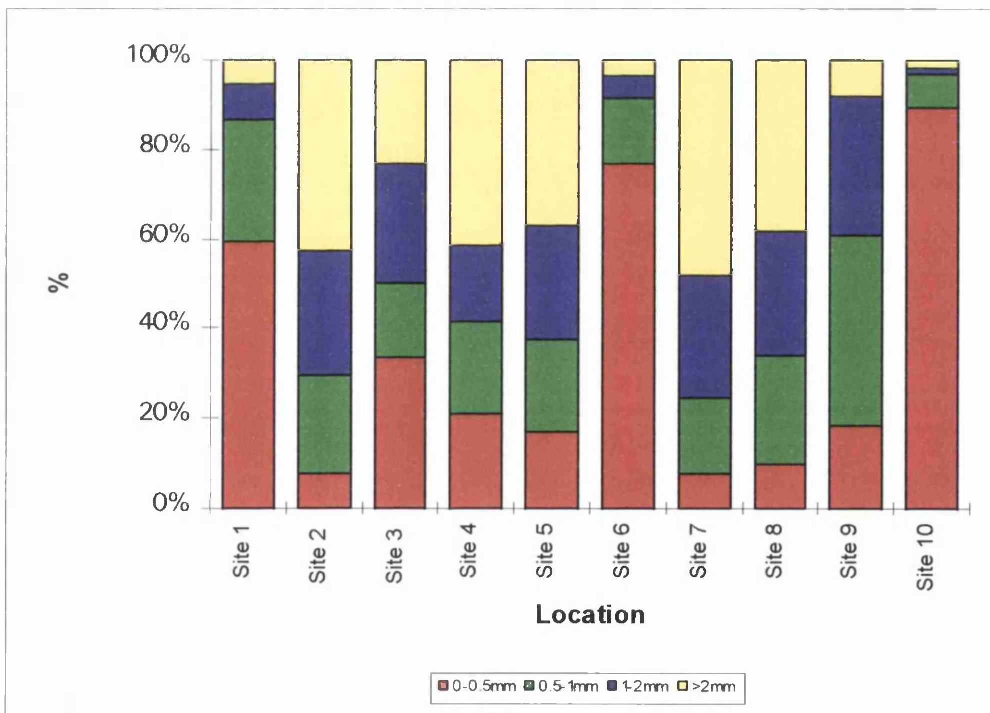


Figure 21: Particle Size Distribution In Annick Water Sediments

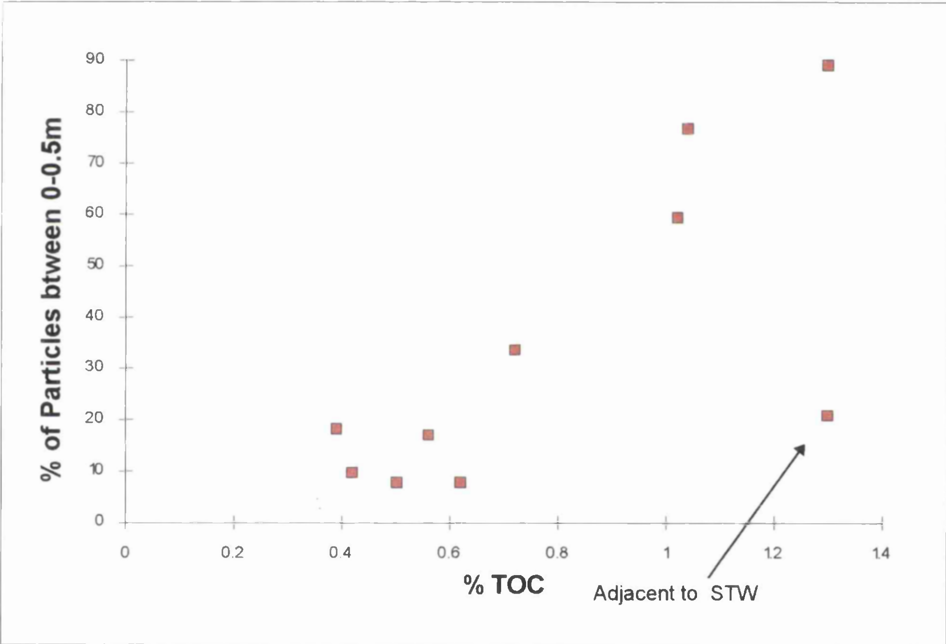


The Annick Water is a small rural upland river, and as a result the organic carbon levels in the sediment are generally low at around 1% TOC.

Figure 22 shows a plot of TOC versus the percentage of particles less than 0.5mm. This demonstrates that TOC and particle size are closely related, with the sampling sites that have a high % of <0.5 mm particles showing the highest TOC levels. This confirms that organic carbon is associated with the smallest particles.

Organic enrichment is indicated in the immediate vicinity of Stewarton STW outlet pipe. This is confirmed by the fact that sediment from this site exhibits a higher organic carbon content than would be expected from the particle size distribution.

Figure 22 % TOC Versus % of Particles <0.5mm Size



Results for the organochlorine analysis of the sediment samples are presented in Tables 99 and 100 and visually in figure 23.

Table 99. Concentration of Organochlorines Pesticides in Sediment from the Annick Water (ug/kg dry weight).

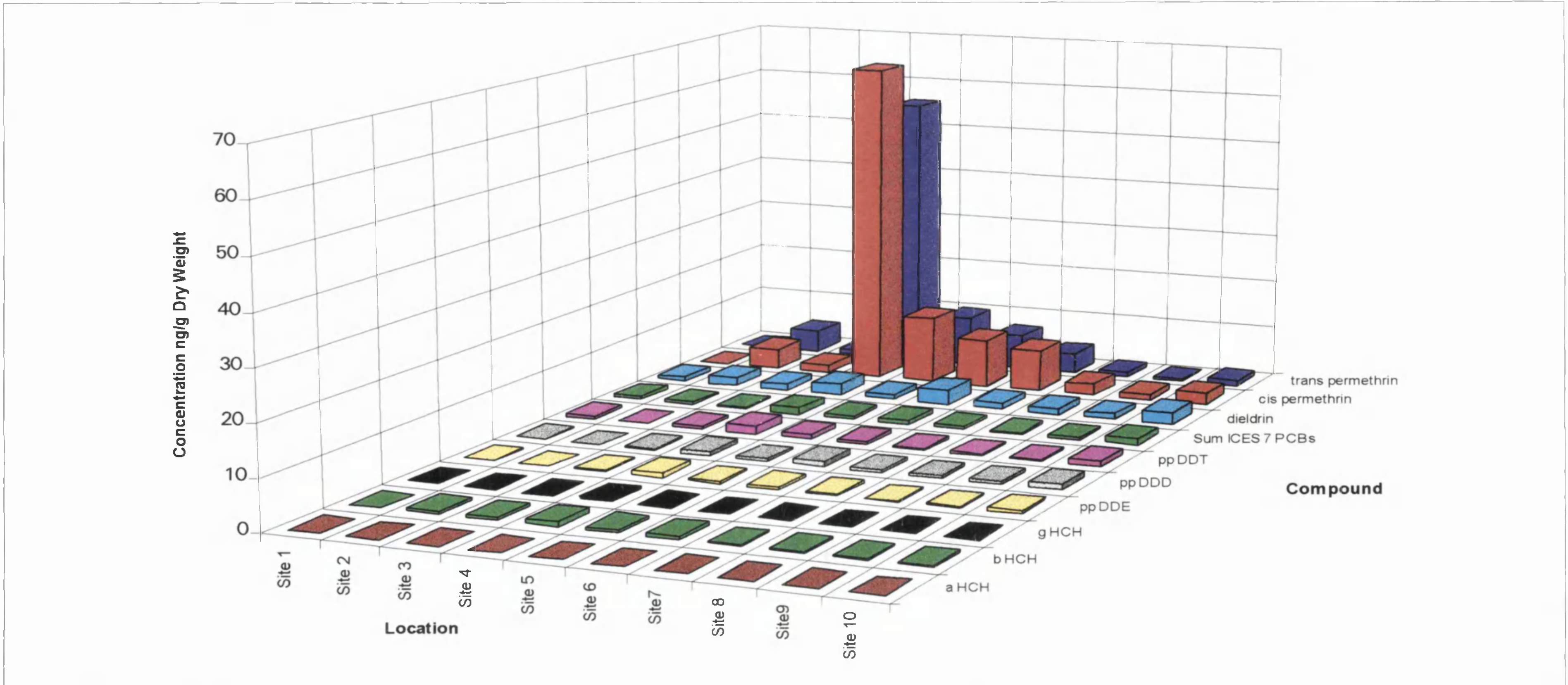
Sample Description	cis permethrin	trans permethrin	dieldrin	pp DDE	pp DDD	pp DDT	α HCH	β HCH	γ HCH
upstream wool processing factory	<0.20	<0.20	0.85	0.12	0.17	0.49	<0.14	<0.1	<0.10
downstream wool processing factory	4.06	4.40	1.58	<0.1	<0.1	<0.1	<0.14	0.56	<0.10
upstream Stewarton STW	1.60	0.94	1.27	0.35	0.31	0.46	<0.14	0.53	<0.10
Stewarton STW at Outlet Pipe	65.88	55.97	2.30	0.95	0.79	1.71	<0.14	0.95	0.37
100M downstream Stewarton STW	13.60	9.94	1.09	0.36	0.37	0.87	<0.14	0.60	<0.10
500M downstream Stewarton STW	9.77	7.05	2.94	0.51	1.04	0.69	<0.14	0.66	<0.10
Chapeltoun	8.58	3.99	1.30	0.33	0.44	0.43	<0.14	0.29	<0.10
Langlands Farm	2.34	0.95	1.19	0.16	0.41	0.41	<0.14	0.29	<0.10
Cunningham Head	1.20	0.45	1.125	0.29	0.55	0.29	<0.14	0.29	<0.10
Perceton	2.37	1.10	2.36	0.67	1.10	1.03	<0.14	0.29	<0.10

Table 100. Concentration of Polychlorinated Biphenyls in Sediment from the Annick Water (ug/kg dry weight)

Sample Description	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 105	PCB 138	PCB 156	PCB 180	Sum ICES 7
upstream wool processing factory	0.16	0.23	<0.05	0.07	0.09	<0.05	0.12	<0.02	0.08	0.67
downstream wool processing factory	0.13	0.18	<0.05	<0.05	<0.05	<0.05	0.05	<0.02	<0.05	0.36
upstream Stewarton STW	0.14	0.18	<0.05	<0.05	<0.05	<0.05	0.1	<0.02	<0.05	0.42
Stewarton STW at Outlet Pipe	0.19	0.24	0.12	0.08	0.2	<0.05	0.39	<0.02	<0.05	1.22
100M downstream Stewarton STW	0.12	0.18	0.08	0.08	0.15	<0.05	0.10	<0.02	<0.05	0.71
500M downstream Stewarton STW	0.27	0.23	0.10	0.08	0.1	<0.05	0.11	<0.02	<0.05	0.89
Chapeltoun	0.13	0.26	<0.05	<0.03	0.05	<0.05	0.17	<0.02	0.05	0.66
Langlands Farm	0.11	0.17	<0.05	<0.05	<0.05	<0.05	0.08	<0.02	0.05	0.41
Cunningham Head	0.12	0.17	<0.05	<0.05	<0.05	<0.05	0.15	<0.02	0.05	0.49
Perceton	0.17	0.18	0.17	0.15	0.19	0.06	0.28	<0.02	0.11	1.25

The pattern of contamination in the sediment samples is similar to the pattern observed in the effluent samples. The nine compounds frequently detected in the effluent were detected in the sediments down stream of the wool processing factory and the STW. Permethrin was again identified as the major contaminant, with trace amounts of dieldrin, DDT, metabolites of DDT, and HCH also identified. This suggests that the STW is the major source of these contaminants.

Figure 23 Distrubution of Organochlorine Contaminants in Sediments from the Annick Water.



4.5.4 Biota

Table 101 Physical Details of Eels Selected for Chemical Analysis.

Site	eel No. 1		eel No. 2		eel No. 3		eel No. 4		eel No. 5		% Lipi	% Wate
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)		
upstream wool processing factory	39.5	102.6	28.1	46.3	29.3	39.2	28.84	39.2	33.1	71.4	12.68	79.5
down stream wool processing factory	28.7	42.84	29.4	42.5	30.2	48.29	29.4	42.5	28	34.37	10.03	70.3
up Stream Stewarton STW	28.8	48.7	30.2	48.3	28.4	39.2	28.2	42.1	29.4	42.5	11.21	65.1
Langlands Farm	38.5	102	31.2	47	37.2	86	32	67.0	31	54	10.54	70.9
Cunningham Head	32	71.4	32.3	53.8	28.7	47.3	32	69.3	31	63	9.94	66.1
Annick Lodge	35.5	93.2	31.5	50.6	37.5	99.8	32.5	75.0	33.7	84	10.27	78.1
Glazert Burn	35	102.6	36	110.6	36	98.6	26	35	25	38	12.13	81.4

Table 102 Concentrations of Pesticides in Eel Tissue ng/g Wet Weight.

Site	cis permethrin	trans permethrin	dieldrin	pp DDE	pp DDD	PP DDT	α HCH	β HCH	γ HCH
up Stream Wool processing factory	<2.0	<2.0	8.4	3.1	<0.4	<0.4	<0.4	<0.4	1.1
down stream Wool processing factory	<3.4	<3.4	407	31.9	<0.7	8.5	<0.7	2.1	11.2
upstream Stewarton STW	<3.1	<3.1	309	32.4	<0.6	9.7	<0.6	1.3	8.2
Langlands Farm	<2.8	<2.8	375	102	<0.6	20.8	2.2	3.2	10.8
Cunningham Head	<3.5	<3.5	400	108	<0.7	23.8	1.5	2.8	9.1
Annick Lodge	<3.3	<3.3	204	97	<0.7	17.3	1.8	1.8	7.7
Glazert Burn	<3.9	<3.9	44.5	87.9	<0.8	9.4	<0.8	<0.8	2.1

Table 103 Concentrations of Polychlorinated Biphenyls in Eel Tissue ng/g Wet Weight.

	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 105	PCB 138	PCB 156	PCB 180	Sum ICES 7
up Stream Wool processing factory	1.62	0.37	0.22	1.48	1.52	<0.1	0.72	<0.1	0.66	6.56
down stream Wool processing factory	7.12	6.75	6.17	12.24	13.42	4.75	9.73	1.02	3.46	58.95
upstream Stewarton STW	3.65	3.52	3.33	5.60	6.23	1.79	3.87	0.47	1.26	27.45
Langlands Farm	4.12	4.46	5.07	6.01	7.98	2.38	6.04	1.14	2.74	36.43
Cunningham Head	4.54	5.21	5.67	7.06	9.11	2.59	7.02	0.75	2.16	40.78
Annick Lodge	4.87	4.35	3.95	6.31	7.94	2.10	5.26	<0.16	2.45	35.07
Glazert Burn	5.18	2.25	1.41	4.02	5.36	1.05	3.80	<0.18	2.5	24.35

The pattern of contamination observed in the eels is significantly different from the pattern observed in sediment and effluent samples.

In contrast to the effluent and sediment samples permethrin was not positively detected in any eel samples.

Dieldrin was the major contaminant detected in the eels, and was positively detected in samples from all sites.

PP DDE was also detected in eels from all the sites sampled and was the major DDT metabolite detected. PP DDT was detected only in eels from sites downstream of the wool processing factory, PP DDD was not detected in any of the samples.

γ HCH was detected in eels from all sites, α and β HCH were detected less frequently and only at sites downstream of the STW.

PCBs were positively detected in eels from all of the sites sampled.

The common pattern for all the contaminants detected is that the highest concentrations are observed in samples obtained from downstream of the STW.

4.6 DISCUSSION

4.6.1 Effluent Data

Of the nine insecticides frequently detected in the effluent from Stewarton STW, permethrin is the only insecticide detected which is currently used as mothproofing agent in the UK. Dieldrin has not been officially used in mothproofing since the mid eighties and has not been used in the west of Scotland since 1978 (Redshaw CRPB pers comm.). α, β and γ HCH, DDT, and metabolites of DDT have never been approved for use as mothproofing agents in the UK (Zabel et al., 1988).

Dieldrin, HCH and DDT have been widely used in sheep dips and veterinary products for the protection of sheep from ticks and sheep scab. Although no longer used for this purpose in the UK these insecticides are still widely used for this purpose in other parts of the world. It is probable that these compounds occur in the effluent as a result of the use of wool imported to the local carpet industry from countries where these compounds are still widely used. Other workers (Shaw, 1994 (a)) have also frequently detected these compounds in effluents from wool treatment processes, with the largest concentrations detected in effluents from wool scouring processes.

HCH is a ubiquitous broad spectrum insecticide with a wide variety of uses, ranging from public hygiene to timber treatment. There are 8 theoretical isomers of HCH. The relative percentage contribution of each of the major isomers to crude or technical HCH is shown below in Table 104.

Table 104 Isomers in technical HCH (Butte 199).

Alpha HCH	60-70 %
Beta HCH	5-12 %
Gamma HCH	10-15 %
Epsilon HCH	6-10 %
Delta HCH	3-4 %

For most of the effluent samples γ HCH was the major HCH isomer detected not α HCH. This indicates that HCH is used as lindane rather than technical grade HCH. This is consistent with a 1979 EC 'Prohibition' Directive (United Nations, New York 1994) banning the use of HCH containing < 99 % of the γ isomer. In both the 24 hour composite samples and the 2 hour composite samples β HCH was detected slightly more frequently and at slightly higher concentrations than α HCH. Their relative abundance α to β HCH in technical HCH suggests that α HCH should have been detected more frequently. Other workers (Cristol, 1947; Butte et al., 1991; Nerin et al., 1992) have reported that β HCH is the most lipophilic and environmentally stable of the HCH isomers. The rate of dehydrochlorination of the HCH isomers has been reported (Cristol, 1947) as increasing from β (unreactive) to δ>>α>γ. β HCH is the most abundant HCH isomer found in man, and domestic sewage is a significant source of β HCH. It is likely that the increased occurrence of β in comparison with α HCH reflects both man as a source and its greater environmental stability.

The relative concentrations of DDT and DDE reflect the history of use of DDT. The parent compound pp DDT was used extensively in central Scotland in the late sixties and early seventies, but its use has been prohibited in the UK since 1984. The DDT observed in the effluent is again likely to come from imported fleeces. PP DDE has not been widely used as insecticide in the UK, however, it is an oxidation product of pp DDT. It is therefore possible that the levels observed derive from secondary sources primarily oxidation of PP DDT in biota including man. The occasional low levels of DDE detected in the effluent therefore may result from sources other than wool processing.

4.6.2 River Water Analysis

Only α HCH and γ HCH were detected in the river water samples. The concentrations detected at both sites were of the same order of magnitude, with the down stream site exhibiting only slightly higher concentrations. This reflects the widespread usage of these compounds. Lindane has consistently been one of the most frequently detected pesticides in surface waters in England, Wales and mainland Europe (NRA, 1995; Council of Europe 1995). Typical background total HCH concentrations in UK surface waters are <10ng/l. The results for the Annick Water are consistent with these levels.

4.6.3 Sediment Data

All of the compounds detected in the sediments are highly lipophilic and it has been suggested that they are likely to be adsorbed very rapidly by suspended particles in a river system (Sharom & Solomon, 1981). The sediment characteristics including grain size and TOC content influence this adsorption process. To minimise the effect of these influences on the results and allow a quantitative estimate of the geographical distribution of contaminant concentrations a method of normalising for these influences is required.

A number of approaches have been applied to compensate for these influences on trace contaminant concentrations. Two of the most common procedures are : (1) sieving the samples and analysing a predefined fraction of the fine sediment (Loring and Nota 1973; Loring 1978, in Loring and Rantala 1992) and (2) using geochemical normalisers, such as the aluminium content (Duinker, 1981; Loring and Rantala 1992). These techniques have largely been used for trace metal normalisation. There are few examples given in the literature of normalisation techniques for organic contaminants. The main approaches again are: (1) analysis of a defined portion of the sediment or (2) normalisation with organic carbon. Results in one of the few studies to compare a variety of normalisation methods (Klammer *et al* ., 1990) recommended the use of <63 um fraction for sediments of similar particle size distribution. Lohse (1991) has shown that a large portion of the total contaminant load is associated with coarse particles in sandy sediments. Delbeke *et al* (1990) noted that PCBs were preferentially associated with lipids in marine sediments.

Sediments from the Annick Water exhibit a wide variation in particle size distribution with the less than 0.5mm fraction accounting for between 7 and 76 % of the total sediment content. For areas with such a wide range of sediment particle sizes it was thought that normalising by analysing a defined portion (e.g. <63um fraction) of the sediment would not be appropriate. This is because from a toxicological point of view organisms are exposed to the complete range of particles which are ecologically important not a small fraction of the sediment. Organic carbon has been shown to be closely linked to particle size distribution and therefore it was selected as the most appropriate normaliser for sediments from the Annick Water.

Many studies on the partitioning of organic contaminants between dissolved and particulate phases have reported the important role of organic matter as a substrate for adsorption (Zhou et al 1995, Karichoff et al 1979). In addition deposit -feeding animals meet their nutritional requirements from the organic fraction of ingested sediment (Lopez and Levinton, 1987). Therefore from both a geochemical and toxicological point of view it is more appropriate to normalise with organic carbon rather than a defined portion of fine sediment.

All the sediment data were therefore normalised to 1% TOC. This is presented in Tables 105 and 106.

Table 105. Concentration (ng/ g dry weight) of Permethrin Normalised on 1% TOC in Annick Water Sediments.

Sample Description	cis permethrin	trans permethrin	Total permethrin	cis : trans permethrin Ratio
upstream wool processing factory	0.25	0.36	0.61	41:59
downstream wool processing factory	8.12	8.79	16.91	48:52
upstream Stewarton STW	2.22	1.31	3.53	63:37
Stewarton STW at outlet pipe	50.67	43.05	93.73	54:46
100m downstream Stewarton STW	24.28	17.74	42.03	58:42
500M downstream Stewarton STW	9.39	6.78	16.18	58:42
Chapeltoun	13.84	6.44	20.28	68:32
Langlands Farm	5.58	2.26	7.84	71:29
Cunningham Head	3.08	1.16	4.24	73:27
Perceton	1.82	0.84	2.66	68:32

The organic carbon normalised results for permethrin are also presented graphically in Figure 23. The spatial distribution of organic carbon normalised permethrin concentrations in the sediment indicate two point sources of contamination for permethrin. The major source is the STW but another source is indicated at the site immediately downstream of the wool processing factory. This second input may represent spillages and natural run off from the factory site. Excluding the influence of this input, the overall picture is of a decline in contamination downstream from the STW.

Table 106. Concentration (ng/g dry weight) of Contaminants Normalised on 1% TOC in Annick Water Sediments.

Sample Description	dieldrin	Total DDT	Total HCH	Total PCB (Sum ICES 7)
upstream wool processing factory	0.8	0.8	<0.3	0.7
downstream wool processing factory	3.2	<0.6	1.1	0.7
upstream Stewarton STW	1.8	1.6	0.7	0.6
Stewarton STW at outlet pipe	1.8	2.7	1.0	0.9
100m downstream Stewarton STW	2.0	2.9	1.1	1.3
500M downstream Stewarton STW	2.8	2.2	0.6	0.9
Chapeltoun	2.1	1.9	0.5	1.4
Langlands Farm	2.8	2.3	0.7	1.0
Cunningham Head	3.1	2.9	0.7	1.3
Perceton	1.8	2.2	0.2	1.0

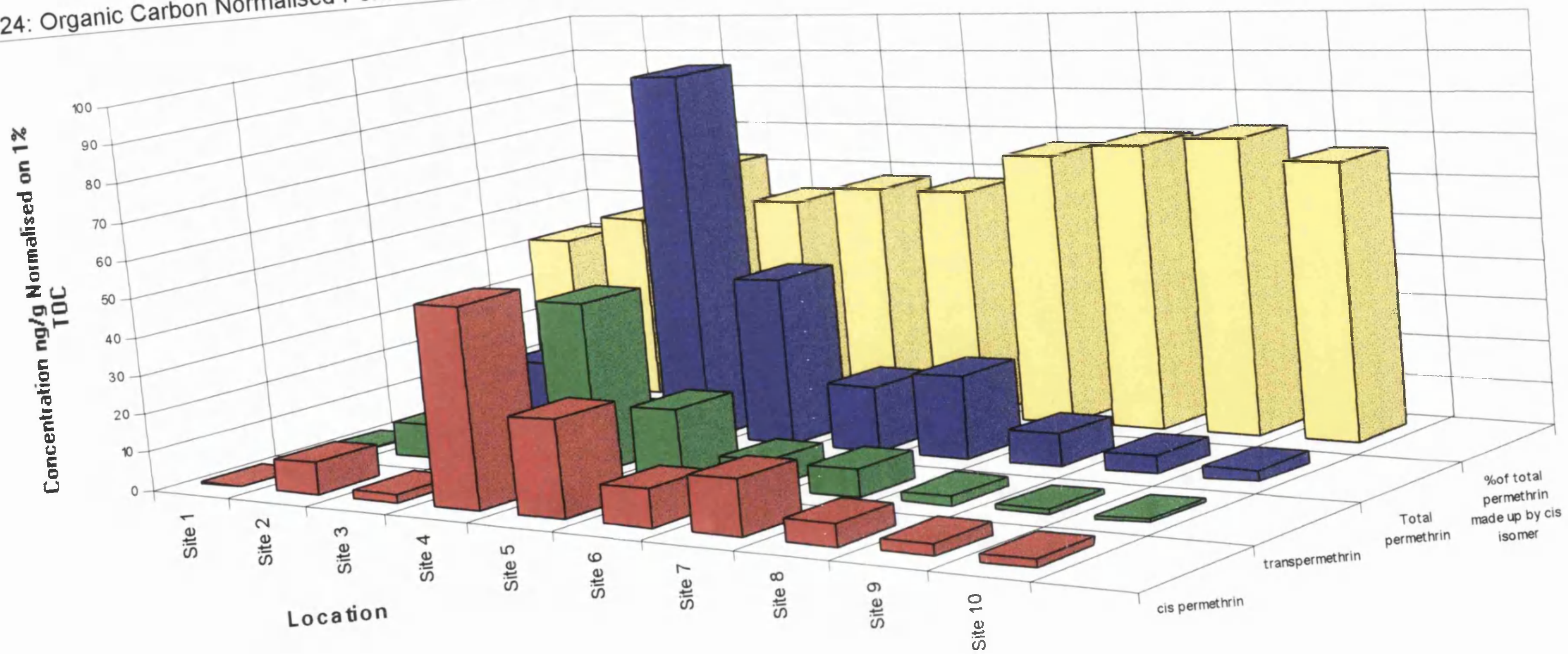
Technical permethrin used in mothproofing contains the isomers cis and trans permethrin in the ratio 40:60 (Zabel, et al 1988). In the effluent, a mean ratio of 44:56 was observed. This is very similar to the ratio of the technical product and agrees with the findings of other studies (Rogers, et al 1989) which show results for the isomers were in the ratio of 40:60 in a variety of UK sewage sludges.

Close to the STW, the sediment bound permethrin is found in the ratio 54:46, and this ratio gradually changes to 68:32 at increasing distances from the discharge. This may be explained by the faster degradation of the trans isomer as observed by other workers (Gambrell, et al 1984, Kirk, et al 1989) who report that the degradation of the trans isomer proceeds much more rapidly than the cis isomer.

Dieldrin, DDT, metabolites of DDT, HCHs and PCBs were all detected at much lower concentrations in the sediment. As a result of the low levels of contamination interpretation of spatial trends is more difficult for these compounds, however, the results indicate that the wool processing factory and STW are both sources of contamination.

The levels of dieldrin recorded in the sediment are of the same order of magnitude as those detected in a number of rivers in England (House, et al 1991) and are orders of magnitude lower than the concentrations of 20-430 ug/kg (Brown, 1979) reported in rivers receiving discharges from the textile industry in the late 70s. When normalised against % TOC the levels remain reasonably constant throughout the catchment.

Figure 24: Organic Carbon Normalised Permethrin Concentration (ng/g dry weight 1% TOC)



A degradation pattern of pp DDT to pp DDD is observed in the sediments at increasing distance from the discharge point. In sediments adjacent to the STW outlet pipe, pp DDT contributes significantly to the total DDT concentration but its contribution to the total DDT levels decreases in the lower reaches of the river. In the far downstream stretch of the river at Perceton, pp DDD is the major DDT metabolite. pp DDE was detected in all the sediments downstream of the STW but its percentage contribution to the total DDT is reasonably constant in all of the sediments. This is consistent with degradation pathways reported by other workers. In sediments and soils DDT is readily degraded abiotically by iron porphyrins and micro-organisms to DDDs (Castro 1964; Zoro et al 1974; Baxter 1990; Pereira et al.,1996). In biota the major degradation pathway is dehydrochlorination to DDE (Hassall 1990; Pereira et al., 1996). DDE is recalcitrant and does not readily degrade in the environment. The consistent % of DDE in the Total DDT concentration suggests that the DDE derives from secondary sources, primarily oxidation of DDT in biota including man (Kelly & Campbell, 1995). The concentrations recorded are orders of magnitude lower than those reported in rivers in England (House, et al 1991), and suggest that DDT concentrations in the Annick river system are close to baseline values

Hexachlorohexanes are much less hydrophobic than the compounds discussed previously. β HCH was detected at low concentrations in all of the sediments, and γ HCH was detected in the vicinity of Stewarton STW outfall. No α HCH or γ HCH was detected in any of the other sediments. This is consistent with the results from the effluent data and confirms the results from studies in the Rhine delta (Hendriks 1993; Hendriks & Pieters 1993) which have reported the contribution of α, β, γ isomers to the total hexachlorohexane concentration in sediments as 20%, 55%, 25% .
This confirms the greater stability in the environment of β HCH.

PCBs were detected in all of the sediments analysed. The organic carbon normalised PCB concentrations were reasonably constant throughout the entire Annick Water catchment with only a very slight enhancement recorded downstream of the STW. In comparison to marine sediments there is relatively little published information of the environmental concentrations of PCBs in freshwater sediments. Harrad et al ., 1994, estimated the mean total PCB concentration in British freshwater sediments as 26ng/g dry weight. The concentrations recorded in the Annick Water are an order of magnitude lower than this concentration. This reflects the rural nature of the catchment of the Annick Water and suggests that PCBs are not a significant contaminant in the Annick Water.

4.6.4 Biota Data

The pattern of contamination observed in the eels is significantly different from the pattern observed in the effluent or sediment samples.

Only dieldrin, pp DDT, metabolites of DDT, isomers of HCH and PCBs were positively detected in the samples of eel muscle. Permethrin was not detected in any of the samples of eel tissue. This is in direct contrast to the results obtained from the analysis of the effluent and sediment samples for which permethrin was a major contaminant detected in the majority of samples.

There is relatively little published information on the occurrence of pesticides and organochlorine compounds in freshwater fish of Britain. The eel is the only British freshwater species which has been studied in any detail (FWR Nov 1994). Table 106 provides an overview of literature data on the concentrations of the above contaminants in fish from sites within Europe.

The concentrations of contaminants detected in eels from the Annick Water are consistent with the concentrations reported in eels from other sites around Europe and concentrations reported in eels from the Annick Water in 1986.

Dieldrin was the major contaminant detected in the eel tissues, the concentrations recorded in eels downstream of the wool processing factory are substantially higher than those recorded in eels from upstream of the factory or from the Glazert Burn. If the concentrations in eels from the upstream and Glazert Burn sites represent background concentrations for this area, inputs from the wool processing factory and STW have lead to a 10 -50 fold elevation in eel dieldrin concentrations. These results agree with a previous study (MAFF, 1988) of eels from Scottish fresh waters conducted in 1986. This comprehensive study of 33 Scottish rivers found that eels from the Annick Water contained the 4th highest concentrations of dieldrin in Scotland. The mean dieldrin concentration found in eels downstream of the wool processing factory is 340ng/g this is only slightly lower than the concentration of 421 ng/g recorded in 1986 indicating that despite restrictions in the use of dieldrin as a mothproofing agent in the intervening period the concentrations in the eels have only decreased slightly. Comparison of the concentrations of dieldrin recorded in the eels from the Annick Water with those reported from other European studies (see Table 106) confirms that eels from the Annick Water contain dieldrin concentrations in the upper range of those reported.

Permethrin was not detected in any of the samples of eel tissue analysed, given the lipophilic nature of permethrin, the high concentrations recorded in the effluent from Stewarton STW and the high concentrations recorded in the sediments from the Annick Water these results are at first surprising.

Table 106 Concentrations of Organochlorine Compounds in Fish from Sites within Europe.

Fish Species	Location	Year	permethrin (ng/g wet weight)	dieldrin (ng/g wet weight)	DDT (ng/g wet weight)	HCH (ng/g wet weight)	PCB (ng/g wet weight)	Reference
eel	R. Avon at Britford	NA	13-33	-	-	20-50	-	FWR 1994
eel	R. Frome	NA	521	-	-	-	-	FWR 1994
yellow perch	Shaft Creek Canada	1979	<10	-	-	-	-	Kingsbury & Kreutzweise 1979
eel	Survey of 33 Scottish River	1986-1987	-	10-1200	30-1000 DDE 5-3000 DD	ND-10 α ND 1500 γ	-	MAFF, 198
"	Survey of 21 English Rivers	1986-1987	-	10-1000	6-400 DDE ND-300 DDD ND-400 DDT	ND-400 α ND-1 1-200 γ	30-1300 Mean 471	"
"	Survey of 6 N.Irish Rivers	1986-1987	-	9-900	30-60 DDE 5-3000 DD	1-8 α ND-50 γ	4-738 Mean 133	"
"	Survey of 2 Welsh Rivers	1986-1987	-	20-300	10-40 DDE 10-40 DDD 3-10 DDT	ND-2 α ND Β 4-5 γ	29-47	"
"	R.Annick	15/10/86	-	421	211 DDE ND DDD 279 DDT	<1 α ND β 53 γ	-	"
"	R.Clyde	17/10/86	-	120	169 DDE ND DDD 171 DDT	<1 α ND β 67 γ	-	"
"	R.Irvine	15/10/86	-	127	264 DDE ND DDD 937 DDT	<1 α ND β 48 γ	-	"
"	Culroy Burn (Ayrshire)	1/10/86	-	105	633 DDE ND DDD 3037 DDT	<1 α ND β 1492 γ	-	"
Yellow eel	South West Netherlands	1989	-	-	-	-	5-200 CB153	de Boer and Hagel. 1994.
"	Netherlands	1981	-	10-1700	40-1560	10-640 γ	90-13000	"
"	Boven Merwede	1992	-	<0.2	15 DDE <0.2 DDD <0.2 DDT	<1 α <1 β 8 γ	230 CB153 75 CB138 94 CB 180	"
eel	Elbe	1985-88	-	-	97-257 DDE 144 -361 DDT	32-97 α 46 -100 γ	52-144 CB 153 24-62 CB180	"
eel	Shannon Ireland	1973-1974	-	6-13	30-40	6-7 γ	123-160	"

Permethrin is readily metabolised in vertebrates undergoing cleavage of the ester bond and oxidation of the released acid and alcohol fragments (Demoute 1989). Laboratory studies of bioconcentration of pyrethroid insecticides in flow through water systems have shown considerable variation in the bioconcentration demonstrated by different test species and size\age classes. No bioconcentration factors for permethrin in eels could be found, however, bioconcentration factors of between 50 and 3000 have been reported for permethrin in *Lepomis macrochirus* (Blue gill sunfish), *Ictalurus punctatus* (channel catfish), *Pimephales promelas* (fathead minnow) (Leahey 1985). In all of these tests bioconcentration reached a plateau within 3 weeks and on removing the organisms to clean water the residues declined rapidly with a 50% loss within a few days. In laboratory studies of the biomagnification of permethrin in the food chain *Daphinia magna* exposed to permethrin in a static sediment water system were fed to *Lepomis macrochirus* (Blue gill sunfish) as the sole food supply. Throughout the period of the study permethrin residues in the fish were less than 5 ug/Kg, indicating that uptake of permethrin from food is far less important than uptake from water (Leahey 1985).

These tests suggest that permethrin being a lipophilic molecule is readily taken up by organisms, however, metabolism and excretion greatly reduce tissue concentrations. Furthermore on cessation of exposure residue concentrations in tissue decline rapidly. The view that permethrin does not bioaccumulate significantly is supported by the work of Kingsbury and Kretzweiser (1979) who following the application of permethrin to 640 ha of forest in Canada measured permethrin concentration in steams and caged specimens of native *Percaflavescens* (Yellow perch) before the application and at 1,3,5,7,8,14,23, and 36 days after spraying. Concentrations in the stream reached a maximum of 147ug/L 96 hours after the application, no permethrin (<10 ng/g) was detected in the perch throughout the study period.

Permethrin has been reported as having been detected in four eels from the river Avon at Britford at concentrations of between 13 and 33 ug/kg and in a single eel from the river Frome in France at the exceptional concentration of 521 ug/kg (IFE 1994). A possible explanation for this is that these concentrations were the result of the eels exposure to a single exposure to extremely high concentrations of permethrin following a pollution incident. No information was available to support this theory, however, it offers a possible explanation for the apparent discrepancies between these studies.

The concentrations of total DDT recorded in the eels from the Annick Water are approximately a quarter of the concentrations detected in 1986. In the study conducted in 1986 DDT was detected in the eel flesh at slightly higher concentrations than DDE. This high DDT/DDE ratio indicated that the eels had recently been exposed to DDT. The low DDT/DDE ratios detected in this present study from eels collected in 1994 indicate that the eels have not been recently exposed to significant concentrations of DDT. The DDE present in the eel tissue arises from historical inputs of DDT

which in time has been metabolised to DDE or direct accumulation of DDE derived from secondary sources.

The concentrations of γ HCH in eels from downstream of the wool processing factory and STW are approximately a factor of 5 higher than those recorded in eels from the upstream and Glazert Burn sites. This suggests that the discharge of HCH has resulted in a 5 fold increase in total HCH concentrations in the eels from these sites. Although elevated from above background concentrations, the published data summarised in table 106 indicates that these concentrations are low in comparison to the concentrations which have been observed in eels from other Scottish and European freshwaters. When compared with the levels recorded in eels from the Annick Water in the MAFF 1986 survey the total HCH concentration recorded in this study indicate that the levels have decreased slightly. Surprisingly α HCH was not detected in any of the 1986 eel samples, but was detected in the eels from 3 of the sites in this study at levels higher than the detection limit quoted in 1986. This suggests that environmental concentrations of α HCH increased during the intervening period. During the intervening period the use of technical HCH was severely restricted, it is reasonable to assume that these restrictions should have led to a reduction in environmental concentrations of HCH.

In comparison with PCB concentrations recorded in eels from England and Wales in the 1986 MAFF survey the total PCB concentrations recorded in the survey of Annick eels are low. Comparison with PCB concentrations from European surveys of PCBs in eels indicates that the concentration recorded in eels from the Annick Water are amongst the lowest reported for European eels, indicating that there no significant sources of PCBs in the Annick Water Catchment.

4.6.5 Comparison with Quality Standards

The UK government has adopted a different approach to eliminating environmental pollution by dangerous substances from other European Union (EU) countries. The UK approach is to set for each substance an environmental quality standard (EQS) for the receiving water. The European approach is to set limit values (LV) prescribed for the concentration of the substances in discharges. The EQSs are chosen with the aim of protecting all aquatic life. These EQS values are set out in UK regulations and are the mechanisms by which EU directives are incorporated into domestic legislation. The UK EQSs for the compounds which have been frequently detected in the effluent from Stewarton STW are summarised in Table107.

Table 107 UK Freshwater Environmental Quality Standards

Compound	Environmental Quality Standard
Total permethrin	10ng/l as a 95 percentile
dieldrin	10ng/l as an annual average
pp DDT	10 ng/l as an annual average
Total DDT	25ng/l as an annual average
Total HCH	100 ng/l as an annual average

It must be emphasised that these quality standards apply to the receiving water not the effluent discharged. To apply the relevant EQS to the contaminants from the discharge from Stewarton STW it is necessary to calculate the likely concentrations of contaminants in the Annick Water immediately downstream of the STW using the measured discharge concentrations with river flow data.

No flow data is available for the Annick Water in the immediate vicinity of Stewarton STW. Flow data is available from Dreghorn (C.R.P.B., 1985) immediately prior to the Annick's confluence with the River Irvine. The flow of the Annick Water at Stewarton was estimated by ratioing the catchment areas above Stewarton and above Dreghorn to the flow of the Annick at the gauging station at Dreghorn. Using this method the flow of the Annick Water at Dreghorn was estimated as 0.56 flow at Dreghorn.

The maximum and dry weather flow of Stewarton STW are 0.084 and 0.028 cumecs respectively (CRPB 1994). Using these flows and the estimated flows of the Annick Water at Stewarton the average and worst case dilutions received by the effluent from Stewarton STW on entering the Annick Water were calculated as 22 and 2. The average dilution was estimated as the average flow of the Annick at Stewarton divided by the maximum permitted flow from the STW. The worst case dilution was estimated by the 95 % exceedence flow of the Annick at Stewarton divided by the maximum permitted flow from the STW. Combining these dilutions with the average and maximum concentrations recorded in Stewarton STW effluent, estimates of the average and maximum concentrations of contaminants in the Annick Water immediately D/S of the STW were calculated. These estimates are listed in Table 108.

Table 108 Estimated Concentrations of Organochlorines in the Annick Water.

Compound	Estimated Average Concentration (ng/l) in Annick Water immediately D/S Stewarton STW	Estimated Maximum Concentration (ng/l) in Annick Water immediately D/S Stewarton STW
cis permethrin	15.4	1070
trans permethrin	14.9	830
Total permethrin	30.3	1900
dieldrin	0.3	5.0
pp DDE	0.3	9.65
pp DDD	0.1	1.35
pp DDT	1.1	51
Total DDT	1.5	67
α HCH	0.1	3.2
β HCH	0.5	18.45
γ HCH	2.5	108
Total HCH	3.1	130

Both in average and adverse conditions the estimated concentrations of permethrin in the Annick Water immediately downstream of the STW would be in excess of the EQS. In average conditions the estimated permethrin concentration was 3 times higher than the EQS, in adverse conditions it is estimated that concentrations of permethrin in the Annick Water could be 200 times higher than those permitted by the EQS.

The concentrations of dieldrin estimated in the Annick Water both in average and adverse flow conditions are well below the annual average concentration of 10 ng/l allowed by the EQS.

The estimated concentration of pp DDT in the Annick Water immediately downstream of the STW is lower than the level of 10 ng/l allowed by the EQS. In the extreme conditions of high concentrations of DDT in the effluent and low river flows the concentration could be as high as 51 ng/l. However, as the EQS is expressed as an annual average there is only a very slight risk of it being breached for pp DDT. This suggests that the concentrations of pp DDT in the effluent from Stewarton STW present only a slight risk to the fauna of the Annick Water.

The estimated average concentration of total DDT type compounds in the Annick Water was an order of magnitude lower than the concentrations permitted by the EQS. In adverse conditions the concentrations of total DDT type compounds could exceed the EQS by a factor of 3, however, as the EQS is expressed as an annual average there is only a slight risk of it being breached for DDT type compounds.

The estimated average concentrations of total HCH in the Annick Water were 30 fold lower than the EQS. In adverse conditions it is estimated that the EQS may be marginally exceeded. The EQS is expressed as an annual average, therefore there is only a very slight risk that it would be exceeded.

These calculations suggest that the permethrin concentrations in the effluent from Stewarton STW pose a serious threat to the fauna of the Annick Water immediately downstream of the STW. The concentrations of pp DDT, total DDT type compounds and HCH pose only a slight risk, and dieldrin does not pose a significant risk to the fauna of the Annick Water.

Permethrin, dieldrin, DDT, and DDT type compounds were not detected in the river monitoring samples from the Annick Water downstream of the STW at Chapeltoun. HCH compounds were only infrequently detected and the concentrations were an order of magnitude lower than EQS. This indicates that the EQS for permethrin, dieldrin, DDT, DDT type compounds and HCH is being met at Chappeltoun.

An alternative to setting EQS or effluent LVs is to derive and set Sediment Environmental Quality Standards (EQS_{Sed}) to protect the ecology of the receiving watercourse. The UK has not set EQS_{Sed}, however, the United States Environmental Protection Agency (US EPA), regulatory authorities in the Netherlands, and UK advisory groups (Group co-ordinating sea disposal monitoring) have recently proposed the use of equilibrium partitioning methods to derive sediment quality criteria from established water quality criteria (Shea 1988; Van Der Kooij *et* al., 1991; and Webster & Ridgway 1994). Unfortunately, no single approach in the derivation of sediment quality standards has become uniformly accepted. The theory underlying a number of the common approaches to the derivation of EQS_{Sed} using the equilibrium partitioning approach is discussed below with particular reference to permethrin.

Concentrations in waters and sediments are related through a partition coefficient :

$$K_{sw} = C_s / C_w$$

Where K_{sw} =solids-water partition coefficient

C_s = concentration in solid phase (mg/Kg)

C_w = concentration in water phase (ug/l)

Using the equilibrium partitioning approach the assumption is made that the critical factor in sediment toxicity is the concentration of the contaminant in the interstitial water.
The sediment standard is, therefore, taken as the concentration in the sediment, in equilibrium with the interstitial water, that does not give rise to a concentration in the water that would breach the water quality criterion for that contaminant.

The solids water partition coefficient for a particular compound can be obtained from experimental measurements and from the literature. The concentration in the water is set at the water quality criterion for that compound. The previous equation can now be rearranged to obtain a EQS_{Sed} for a particular compound.

For low organic carbon (0.89 %) freshwater sediments which are similar to those found in the Annick Water previous workers (House *et al*., 1991) have calculated Log K_{sw} as 1.5 and 2.0 for cis and trans permethrin respectively. Using a mean value of Log K_{sw} = 1.75 and the EQS of 0.01 ug/l the EQS_{Sed} is estimated as 0.56 mg/Kg.

Other workers measured Log K_{sw} as 2.59 (Sharom & Solomon, 1981) and 2.3 (Hill, 1989) in a 43 % organic matter lake sediment and a soil respectively. Using these values the EQS_{Sed} is estimated as 3.89 and 2.0 mg/Kg dry weight.

Numerous workers have demonstrated the significance of organic carbon in controlling the sorption of hydrophobic compounds to soils and sediments (Goring, 1962; Hamaker & Thompson, 1972; and Lambert *et al*., 1965 cited in Karickhoff, 1981; Adams *et al*., 1992; Burton & Scott, 1992). This calculation does not account for variations in organic carbon and assumes that the sediments are similar both chemically and in organic carbon content.

To overcome this limitation, a variety of methods utilising the organic content of the sediment have been proposed. Two of these methods are outlined below:

Method 1

Van Der Kooij et al 1991 demonstrated that a sediment water quality criterion could be expressed by the following equation.

$$C_s = C_w * TOC * K_{ow} * 10^{-0.21}$$

Where C_s = concentration in solid phase (mg/Kg)

C_w = concentration in water phase (ug/l)

TOC = Total Organic Carbon expressed as a fraction (e.g. 1% is 0.01)

K_{ow} is the octanol water partition coefficient.

K_{ow} for permethrin has been reported as between 3.49 and 6.6 (Noble,1993) with a median value of 6.24. Using these values and the freshwater EQS of 0.01ug/l the EQS_{Sed} is estimated as between 0.19 and 245 mg/Kg with a median value of 107 mg/Kg for a 1 % TOC sediment.

Method 2

More recently Webster & Ridgway, 1994 have reported that EQS_{Sed} can be evaluated using the following equation.

$$C_s = K_{oc} * C_w * TOC$$

Where C_s = concentration in solid phase (mg/Kg)

K_{oc} = solids-water partition coefficient normalised to organic carbon i.e. $K_{oc} = K_{oc}/$ fraction TOC

C_w = concentration in water phase (ug/l)

TOC = Total Organic Carbon expressed as a fraction (e.g. 1% is 0.01).

Using Log_{oc} = 3.5 and 4.1 (House et al 1991) this gives EQS_{sed} of 0.32 and 1.26 ng/g dry weight respectively.

Using the approaches outlined above EQS_{Sed} have been calculated for the compounds detected in the Annick Water sediment using a range of published partition coefficients, these calculated EQS_{Sed} are summarised in Table 109. No data is presented for PCBs as at present there is no UK EQS for PCBs in water.

Table 109 Range of Derived Sediment Environment Quality Standards

Compound	Log K_{ow}	Log K_{oc}	EQS 1% TOC ng/g dry weight	Method of Calculation
permethrin	6.6 3.49 6.50 5.84 6.24		245 0.19 195 43 107	EQS_{sed} from logK_{ow} As per Van der Kooij
		3.5 (cis) 4.1 (trans)	0.32 1.26	Measured Log K_{oc} EQS_{sed} as per Webster
	6.6 3.49 6.5 5.84 6.24	6.18 3.10 6.08 5.43 5.83	164 0.128 121 26.9 66.8	Log K_{oc} estimated as per Karickhoff EQS_{sed} calculated as per Webster
MEDIAN			55	
dieldrin	4.54 4.32 5.4		6.41 3.86 46.46	From logK_{ow} As per Van der Kooij
		3.2 4.36	0.48 6.84	Measured Log K_{oc} EQS_{sed} as per Webster
	4.54 4.32 5.4	4.144 3.926 4.995	1.393 0.843 9.89	Log K_{oc} estimated as per Karickhoff EQS_{sed} calculated as per Webster
MEDIAN			5.1	
pp DDT	6.38 6.2 6.19 6.91		148 98 95 501	From logK_{ow} As per Van der Kooij
		5.20 5.38	16 24	Measured log K_{oc} EQS_{sed} as per Webster
	6.38 6.2 6.19 6.91	5.96 5.79 5.78 6.49	92 61 60 308	Log K_{oc} estimated as per Karickhoff EQS_{sed} calculated as per Webster
MEDIAN			94	
Lindane	3.72 3.66		3.23 2.82	From logK_{ow} As per Van der Kooij
		3.5 3.29 3.72	3.16 3.9 5.25	K_{oc} measured EQS_{sed} as per Webster
	3.72 3.66	3.33 3.27	2.15 1.88	Log K_{oc} estimated as per Karickhoff EQS_{sed} calculated as per Webster
MEDIAN			3.2	

The equilibrium partitioning (EP) approach relies heavily on experimental partition coefficients. The measurement of partition coefficients is a complex and time consuming process and a large number of methods have been used to derive these coefficients. To date no single method has been universally accepted and as result there are wide discrepancies in the literature values for these partition coefficients. The lack of accepted partition coefficient data is more acute for lipophilic

compounds such as permethrin and this accounts for the extremely wide discrepancies in the calculated sediment quality standards for these compounds.

In addition to problems with obtaining high quality physical constant data researchers have recently begun to question the scientific principles on which the EP approach is based.

The EP approach relies on the assumption that the toxicity of sediments is derived solely from contaminants partitioned into the pore water. Recent studies suggest that this may be an over simplification (Hoke *et al* 1994; Ankley *et al* 1994; Hoke *et al* 1995), and contaminants bound to ingested sediments may be equally important. In addition the majority of water quality criteria have been derived from toxicity data for pelagic fauna, it cannot be assumed that these species have a similar toxic response to benthic species. There is only a limited amount of toxicity data for pelagic organisms and even less data for benthic organisms. Test species are usually bred in captivity to conduct toxicity tests and some of these test species e.g. *Daphnia* are often not the most sensitive to contaminants. The populations of these species used for these tests as they have survived being bred and handled may be more resistant than the general population. The majority of toxicity tests are short term acute tests and do not account for chronic effects such as carcinogenic, oestrogenic, and bioaccumulative effects.

Despite these difficulties the setting of sediment quality criteria is desirable as they should provide a convenient method of using measured concentrations of contaminants to make assessments of possible adverse biological effects. In order to become widely used these criteria must be accepted by the public, dischargers and the regulatory bodies. To achieve this, these criteria must be based on sound scientific principles. The procedures used to derive the standards must be unambiguous and well documented. Data used in the derivation of the quality criteria should be of a high quality and beyond refute.

At present the author does not feel that these conditions are met. The lack of high quality equilibrium coefficient data is a major draw back particularly for lipophilic compounds such as permethrin. It is therefore the authors opinion that at present the equilibrium approach is not suitable for the setting of sediment quality criteria for most contaminants. For the more water soluble compounds such as lindane the equilibrium data currently available is more acceptable and EQS_{Sed} can have a role to play in indicating levels of concern.

Comparison of the median EQS_{Sed} calculated in Table 109 with the 1 % organic carbon normalised concentrations of contaminants listed in Table 105 indicates that the total permethrin concentrations close to the STW outfall are greater than the median EQS_{Sed}. This suggests that these concentrations are likely to be acutely toxic to the fauna of the Annick Water. The concentrations of permethrin recorded in the sediments immediately downstream of the wool processing factory and at Chappeltoun are approximately half and a third of the median EQS_{Sed}. These concentrations are

very close to the EQS_{Sed}, it is therefore difficult to draw conclusions on their toxicity to the fauna of the Annick Water. The concentrations recorded in sediments from the downstream sites such as Cunningham Head and Perceton were an order of magnitude lower than the median EQS_{Sed}, this suggests that in fact permethrin is unlikely on its own to be toxic to the fauna of the Annick Water at these sites.

The concentrations of dieldrin in the sediments at all the sites downstream of the wool processing factory was approximately half of the median EQS_{Sed}. As the concentrations of dieldrin in the sediments are very close to the calculated EQS_{Sed} no conclusions on the toxicity of the sediment bound dieldrin can be made.

The concentrations of pp DDT recorded in the sediments at all the sites are an order of magnitude lower than the median EQS_{Sed}. This suggests that sediment bound pp DDT and DDT type compounds are also not significant toxicants in the Annick Water system.

The concentrations of total HCH were between a third and a fifth of the median EQS_{Sed} at all sites downstream of the wool processing factory. As these concentrations are close to the median EQS_{Sed} then no firm conclusions on the toxicity of sediment bound HCH can be made.

The UK and European countries do not at present have environmental quality standards for the concentrations of persistent contaminants in biota, however, the UK and a number of other countries have standards and/or guidance values for contaminants in Fish for the assessment of possible hazards to human health. A selection of European Standards/guidance values for fish are summarised in Table 110.

Table 110 A Summary of European Standards/Guidance Values for Contaminants in Fish for Human Consumption. (JMP 17/3/10-E 1992, MAFF 1993)

Country	permethrin ng/g wet weight	dieldrin ng/g wet weight	DDT ng/g wet weight	HCH ng/g wet weight	PCB ng/g wet weight
UK	-	200 MRL	5000 MRL	2000 γ MRL	-
Germany		-	2000	500 $\alpha+\beta$ 2000 γ	80 CBs 28, 52, 101, 180 100 CBs 138,153
Norway	-	-	2000	50 $\alpha+\beta$ 200 γ	1000
Netherlands	-	100	500	50 $\alpha+\beta$ 50 γ	100 CBs 28, 138, 153 40 CBs 52 80 CBs 101,118 120 CB 180
Sweden	-	-	5000	200 $\alpha+\beta+\gamma$	2000

MRL - Maximum Residue Limit in meat fat (FAO/WHO,1987).

The concentrations of dieldrin recorded in eels from the Annick Water down stream of the wool processing factory , upstream of Stewarton STW , Langlands Farm, Cunningham Head and Annick

Lodge were in excess of both the UK and Netherlands standards/guidance values for dieldrin concentrations in meat and fish products. This suggests that regular consumption of eels caught from these locations could have human health risks. The concentrations of dieldrin in the tissues of eels from these locations may also present a potential threat to fish eating birds and to wild mammals such as otters.

The concentrations of DDT, metabolites of DDT, isomers of HCH and PCBs detected in the eels from all of the sites on the Annick Water and Glazert Burn are at least an order of magnitude lower than the strictest European standards/guidance values. This confirms that the concentrations of these contaminants in the eels do not present a risk to human health.

The levels of PCBs and other contaminants in fish which pose a threat to wild mammals such as otters is the subject of considerable debate. Boer (1984) has shown a lowered reproduction rate in laboratory mink on a daily intake of PCBs equivalent to a concentration of 25ng/g wet weight PCB in the food. Mason & Macdonald (1993) have implicated PCB concentrations of 210 ng/g wet weight PCBs in eels from the River Glaven (England) in the death of otters reintroduced into the river catchment. It has also been suggested that PCB levels in areas such as central Scotland (Mason et al 1992) and North East England (Mason 1993) are preventing the repopulating of these areas by otters. More recent studies (Kruuk & Conroy 1996) have suggested that extrapolations between laboratory mink tests and wild otter populations exaggerate the importance of PCBs and persistent contaminants as a limiting factor in the viability of natural populations. Shetland possesses a thriving population of otters, these otters have the highest PCB burdens of otters in Scotland more than double the concentration causing reproductive failure in minks (Kruuk & Conroy 1996). The concentrations of PCBs in otters from Shetland correspond to a PCB concentration of approximately 100 ng/g wet weight in their food (calculated from Kruuks & Conroys data as per Mason 1993). The concentrations of PCBs recorded in eels from the Annick Water are similar to those shown to have reproductive effects on laboratory mink but are considerably lower than the calculated concentrations in the food of Shetland otters. This suggests that PCB concentrations in the Annick are not a major factor in the viability of otter populations in the area.

The indicated fish standards are primarily designed to protect man not the eels or the environment as a whole. Recently the Netherlands have developed a series of ecotoxicological based standards (van der Valk et al 1989) for the assessment of contaminant residues in biota. The JMG have also recently developed a set of arbitrary descriptive guidelines for contaminants in biota (MAFF, 1993, Kelly 1994). These more environmentally relevant standards/guidelines are summarised in table 111.

Table 111. Summary of European Standards/Guidelines for Biota.

Compound	JMG guidelines ng/g wet weight	Netherlands Ecotox. Standards ug/Kg lipid
Permethrin	-	-
dieldrin	200 - 300	800
DDT	500 DDE 500 DDD 500 DDT	Total DDT 400 DDE 125
HCH	50 γ	500 γ
PCBs	JMG Guideline Classification <10 Low 10-50 Medium >50 Upper	200 CBs 28,138,153 80 CBs 52 160 CBs 101,118 240 CBs 180

Surprisingly the environmental standard for dieldrin is slightly higher than the standard for the protection of human health, clearly humans are part of the environment and a standard designed to protect the environment should also protect humans. These standards are often drawn up in an arbitrary fashion, the discrepancy between these two standards underlines the need to apply these standards with caution.

Dieldrin concentrations in eels from the Annick Water are slightly higher than the JMG guideline concentrations but lower than the Netherlands provisional ecotox standards. This suggests that the dieldrin levels are substantially elevated above background and are of concern but have not reached the level where they are acutely toxic.

The concentrations of DDT, metabolites of DDT and isomers of HCH detected in eels from the Annick Water were lower than the JMG guidelines and the Netherlands provisional ecotox standards suggesting that the concentrations detected in the eels do not pose a significant risk. The concentrations of individual PCB congeners detected in eels from the Annick Water were lower than the Netherlands provisional ecotox standards for individual PCB congeners.

Eels from the site downstream of the wool processing factory were in the upper JMP category for total PCB concentrations. Eels from upstream of the STW, Langlands Farm, Cunningham Head, Annick Lodge and the Glazert Burn were in the middle JMP classification. Eels from the Annick Water upstream of the wool processing factory were in the lower JMP classification.

4.6.5 Comparison with Biological Effects

River invertebrate communities are good indicators of water quality (CRPB 1993). Similarly to sediments, invertebrate communities are continuously exposed to changes in water quality over periods of weeks or months. Therefore, they integrate the effects of pollution over time. Clean rivers can support a rich variety of invertebrates, but as organic pollution increases, sensitive groups such as stoneflies and mayflies disappear and tolerant groups such as worms and midge larvae dominate the fauna.

The Clyde River Purification Board collects samples of bottom - living invertebrate organisms twice a year from a network of sites on rivers and streams throughout the Board's area. The invertebrate families present in these samples are identified and the results summarised as two UK standard biotic indices, the Biological Monitoring Working Party (BMWP) and Average Score per Taxon (ASPT) index. This work is carried out by routinely by CRPB biologists.

The BMWP Score is calculated by giving the invertebrates most sensitive to organic pollution (e.g. stoneflies) a score of 10. Other invertebrates are given lower scores depending on their sensitivities. The most tolerant invertebrates (e.g. worms) are given a score of 1. The scores for each family of invertebrate present in the sample are added together to give the BMWP score. There is no fixed upper limit for BMWP scores, unpolluted rivers may have widely differing BMWP scores. A rivers natural capacity to support life is greatly influenced by geology, topography, the rivers physical and chemical character. Unpolluted chalk streams have BMWP scores in excess of 200. Typical values for an unpolluted river similar to the Annick Water are >100 (Doughty, pers comm.).

ASPT is a modification of the BMWP score and is calculated by dividing the BMWP Score by the number of scoring families. This index is less influenced by sampling and seasonal effects than the BMWP score. 10 is the theoretical maximum ASPT score. In practice values greater than 7 are only rarely encountered and 5.5 is representative of a clean river similar to the Annick Water.

A disadvantage of biotic indices such as BMWP score and ASPT is that they respond to factors unrelated to pollution e.g. habitat and physical characteristics of watercourses. To avoid misleading results in national surveys of river water quality CRPB classifies the pollution status of rivers using the River Invertebrate Prediction and Classification System (RIVPACS). This is a computer software package which predicts the invertebrates (and therefore the biotic indices) that would be expected at a given site in the absence of pollution, taking into account the sites physical and chemical features. By comparing the actual community found with that predicted by RIVPACS, environmental quality indices are derived. These indices are banded to produce the broad biological quality classes listed overleaf:

Class	Quality
A	Good
B	Moderate
C	Poor
D	Very Poor

Mean BMWP score, ASPT score and RIVPACS class for the Annick Water in 1993 are shown below in Table 111b. This work was carried out by CRPB biological staff as part of their routine monitoring of the Annick Water.

Table 111b. 1985 Mean BMWP Scores for Annick Water

Site	Grid Ref	Mean BMWP Score	Mean ASPT	RIVPACS Class
upstream wool processing factory	NS 452 484	141	6.54	A
between wool processing factory and STW	NS 410 452	54	4.73	B
Chapeltoun	NS 394 440	28	3.45	D
Perceton	NS 350 405	36	3.66	D

Up stream of the wool processing factory the Annick Water supports a wide variety of invertebrates, this is reflected in its high BMWP and ASPT scores. At this point the Annick Water is of good biological quality and is not significantly impacted by pollution. Downstream of the wool processing factory much lower BMWP and ASPT scores are recorded, and pollution sensitive invertebrates are absent from the water course at this point. The biological quality of the Annick Water has significantly deteriorated and is now of moderate quality. Down stream of the STW at Chapeltoun this deterioration has continued, very low BMWP and ASPT scores are recorded at these sites. Only the most pollution resistant invertebrates are found, at this site the Annick Water is classified as of very poor biological quality. The lower reaches of the Annick Water at Perceton immediately before the Annicks confluence with the Irvine only a marginal improvement in biological quality has been observed. Low BMWP and ASPT scores are recorded and only pollution tolerant invertebrates are found. The Annick Water is still classified as of poor biological quality at this site.

The pattern of invertebrate biological quality data and circumstantial evidence from the perimeter of the wool processing site suggests that leaks, runoff and non-consented discharges from the wool processing factory have a marked effect on the biological quality of the Annick Water. The biological invertebrate quality data also indicates that the discharge from Stewarton STW has a severe impact on the invertebrate fauna of the Annick Water. The Annick Water remains severely impacted until its confluence with the River Irvine.

As discussed earlier while obtaining samples of eels from the Annick Water for chemical analysis CRPB Biology staff carried out fish population studies (counting variety of species and numbers of fish caught at each location). The results of these fish population studies are available in a CRPB internal report (Doughty CRPB 6/10/94). The findings of this study are summarised below in tables 112 and 113.

Table 112. Distribution of Fish Species in the Annick Water: dots indicate relative abundance of each species.

	Site No.							
Species	1	2	3	4	5	6	7	8
Brown Trout	●●	●●	●		●	●	●	●
Salmon		●●	●			●	●	●
Minnow	●●●	●●	●●●	●●●	●●●	●●	●●	●●●
Stoneloach	●	●	●	●	●●	●●	●	●●●
Stickleback			●●	●	●●	●	●	●
Eel	●	●	●		●	●	●	●
Brook lamprey			●	●				

Table 113: Densities of Salmonid fish (per $100m^2$) in the Annick Water.

Site	Trout		Salmon		
	Fry	Other	Fry	Other	Total Salmonid s
1. Annick Water upstream Wool processing factory	3.2	5.8	0	0	9.0
2. Annick Water down stream Wool processing factory	0.6	6.9	1.4	2.7	11.6
3. Annick Water upstream Stewarton STW	0	1.8	0	0.2	2
4. Annick Water Chapeltoun	0	0	0	0	0
5. Annick Water Langlands Farm	0	0.5	0	0	0.5
6. Annick Water Cunningham Head	0	0.3	0.2	0	0.5
7. Annick Water Annick Lodge	0	0.7	0	0.2	0.9
8. Glazert Burn upstream of Langlands farm	0	3.5	0	0.3	.38

The fish community in the Annick Water is typical of a small, fast flowing river in the west of Scotland. Seven fish species were recorded in the Annick Water. The minnow was the most numerous fish in the Annick Water. Trout were present at seven of the eight sites sampled, being absent only at Chapeltoun. However, the numbers of trout were low at all sites below Stewarton. Salmon (fry plus parr) were present at five of the eight sites. However, the densities of salmon at these sites were extremely low with only single specimen caught at all the sites except the B778 Road Bridge. These results show that Salmonid fish are rare in the Annick Water downstream of Stewarton. Numbers decline appreciably between the wool processing factory and the STW . The salmon population in the Glazert Burn is considerably greater than the Annick Water.

There is little evidence of Salmonids spawning in the Annick below Stewarton, although numerous areas of suitable habitat do exist. If significant spawning does take place the lack of fry in the Annick Water indicates that the fry are subject to a very high mortality. It is likely that the sparse Salmonid population present in the Annick Water is sustained by stocking or recruitment from tributaries.

Even pollution tolerant species such as eels were absent from sites close to the STW such as Chapeltoun.

The pattern of Salmonid distribution and abundance strongly suggests that the discharge from Stewarton STW has a severe impact on Salmonid populations in the Annick Water. This is in agreement with the results from the invertebrate fauna studies. The fish population studies do not suggest that leaks or discharges from the wool processing factory are having a toxic effect on the fish of the Annick . This is in contrast to the invertebrate fauna data which suggests that there is an impact from the wool processing factory. A possible explanation for this is that the invertebrates are more sensitive to the mothproofing chemicals and the decreased fish populations in the lower stretches of the Annick are due to removal of food sources rather than a direct toxic effect. A large number of small streams join the Annick close the wool processing factory these streams may provide populations of both fish and invertebrates to restock the Annick Water. This may explain the abundance of fish close to the wool processors, and account for the apparent discrepancy between the fish and invertebrate data.

The concentrations of contaminants routinely detected in the Annick Water up and downstream of the STW were orders of magnitude lower than the EQSs. These biological effects are far greater than can be explained by these concentrations. Comparison of the concentrations of contaminants detected in the sediments with EPA derived EQS_{Sed} suggests that permethrin and dieldrin are the contaminants present in the sediments most likely to exert a toxic effect on the Annick Water system.

There is very little information on the toxicity of sediment bound organic contaminants to freshwater life.

At present the author is not aware of any published sediment toxicity on dieldrin. However, a number of studies have been published on the toxicity of Endrin to North American invertebrates. Endrin is a member of the cyclodiene class of insecticides and possesses similar chemical properties and toxicity to dieldrin. In the absence of published sediment toxicity data for dieldrin the published data for Endrin has been considered as the best available alternative.

In the amphipod _Hyalella azteca_ 50% mortality has been reported (Nebeker _et al_ 1989) after 10 day exposure to a 3% organic carbon sediments containing 4400ng/g (dry weight) Endrin.
In sediments spiked with Endrin at 995 000 ng/g (dry weight) 50 % mortality of the Oligochaete _Limnodrilis hoffmeisteri_ has been reported (Keilby _et al_ 1988) over a 96 hour test period. In the same study in a non lethal test, sediment avoidance was reported in _Limnodrilis hoffmeisteri_ at Endrin sediment concentrations of 59000 ng/g. Limnodrillis are freshwater worms, they are widely distributed in poorly oxygenated waters and are considered tolerant of organic pollution (Barnes 1980).

Conclusions drawn from such a limited set of toxicity data must be treated with extreme caution. However, the concentrations of dieldrin recorded in the Annick Water are more than 1000 times

lower than those of Endrin shown to have a toxic effect on amphipods in laboratory tests. This suggests that sediment bound dieldrin is not a major invertebrate toxicant in the Annick Water.

Recent work (Vine, 1993) on sediment toxicity tests of permethrin to Ephemera dancia (mayfly nymph) have reported narcosis at 72.5 ng/g (dry weight) and 53% mortality at 389 ng/g. In Hexagenia rigida (mayfly nymphs) 100% mortality after a 7 day exposure to an average concentration of 50 ng/g (Dry Weight) has been reported (Friesen et al 1983). Concentrations of approximately 40 ng/g (wet weight) were recorded in freshwater pond sediments after 2 applications of 17.5 g/ha permethrin (Kingsbury & Kreutzweiser 1979), this resulted in a substantial reduction in the invertebrate population and it took six weeks for the population to recover to pre-treatment levels.

The sediment survey shows that the sediment of the Annick Water below the STW is contaminated with permethrin. The concentrations immediately adjacent to the works are similar to those shown to have toxic effects, while further down stream the concentrations are below toxic levels. The invertebrate fauna data show clear toxic effects both at the higher and lower contaminant levels. This enhanced toxicity may be explained by consideration of the isomeric ratios of permethrin detected in sediments at the sampling sites.

The technical product used in mothproofing contains cis and trans permethrin in the ratio 40:60. In the effluent , a mean ratio of 44:56 was observed. This is very similar to the ratio of the technical product and agrees with published data (House et al., 1991; Rogers et al .,1989). In the sediment close to the STW, the permethrin is found in the ratio 54:46 but this gradually changes to 68:32 at increasing distances from the discharge. This change in cis : trans ratio is explained by the faster degradation of the trans isomer. This is supported by other workers (Gambrell et al 1984, and Kirk et al 1989) who have reported that the degradation of the trans isomer proceeded much more rapidly than the cis isomer in soils. Biological systems are very sensitive to the sterochemistry of molecules. Other workers (Cremlyn 1991) have demonstrated that changing the cis to trans ratio of permethrin can have a dramatic effect on the toxicity of technical grade permethrin. Table 114 taken from (Cremlyn 1991) illustrates the effect of changing the cis/trans ratio of permethrin on the LD50 values in mg/Kg for rats.

Table 114 The Effect of cis:trans Ratio on the Toxicity of Permethrin to Rats.

cis : trans permethrin Ratio	LD50 mg/Kg, Oral to Rat
20:80	6000
40:60	1250
80:20	220

It is widely recognised (Leahey 1985) that the cis isomers are the more insecticidally active of the permethrin isomers. However, the data in the above table suggests a much greater effect than a simple difference in toxicity between cis and trans isomers. The toxicity of the 80:20 mixture cannot be explained solely on the grounds of the cis isomer being more toxic, the toxicity demonstrated by this mixture is due to synergistic activity. This synergistic activity is well known for permethrin (Cremlyn 1991, Hassall 1990, and Leahey 1985) Sesame seed oil is a well known synergist for permethrin. A mixture of permethrin and Sesame seed oil is 7 times more active than permethrin on its own (Cremlyn 1991). The limited number of sediment toxicity tests that have been conducted on permethrin have all been carried out using permethrin with a 40:60 cis:trans ratio over a limited time period. These conditions will not account for degradation of the cis isomer and the corresponding increase in the toxicity of the permethrin due to synergistic activity. Permethrin in the environment and particularly the permethrin bound to sediments in the lower reaches of the Annick Water will contain a higher percentage of cis permethrin than the technical permethrin used in toxicity tests. In addition to the synergism caused by the changing cis:trans ratio other chemicals present in the environment or occurring naturally in the river could be adding to the synergistic effects on the sediment bound permethrin. Organophosphorus compounds are also excellent pyrethroid synergists interfering with the microsomal oxidation of the pyrethroids and hence reducing their metabolism. The organophosphorus insecticides diazinon, and propetamphos are extensively used as the active ingredients of sheep dip treatments. Both of these pesticides have been detected in the Annick Water (Pirie unpublished results).

In addition to synergistic activity permethrin along with most pyrethroids has a negative insecticidal temperature coefficient. That is, permethrin is more toxic at lower temperatures (Leahey 1985, Cremlyn 1991). Large numbers of toxicity tests are carried out between 10 and 25 °C, in the winter months water temperatures may be considerably lower than this. These lower temperatures may lead to greater toxic effect than would be predicted from laboratory based toxicity tests carried out at room temperature.

It is suggested that the negative insecticidal temperature coefficient of permethrin along with synergistic behaviour of the sediment bound permethrin accounts for the discrepancy between theoretical values and the observed toxic levels in the field.

4.7 Conclusions

Organochlorine compounds were detected in the effluent from Stewarton STW, the Annick Water, sediments from the Annick Water and eels from the Glazert Burn and Annick Water.

Permethrin, isomers of HCH, dieldrin, DDT and metabolites of DDT were detected in the effluent from Stewarton STW. Permethrin and isomers of HCH were the compounds detected most frequently and at the highest concentrations.

The concentrations of permethrin detected in the effluent from Stewarton STW were likely to cause a breach of the freshwater environmental quality standard for permethrin in the Annick Water immediately downstream of the STW.

It was predicted that due to their lipophilic properties all of the organochlorine compounds detected in the effluent from Stewarton STW would be rapidly adsorbed onto suspended particles in the Annick Water. It was also predicted that adsorption would ensure that the EQS for permethrin was breached only for a short stretch of the Annick Water. This prediction was confirmed by the analysis of samples from the Annick Water at Chappeltoun. Isomers of HCH were the only compounds detected and the concentrations detected were comparable to those reported for other rivers in Europe.

Permethrin, dieldrin, HCH isomers, DDT, DDT metabolites and PCBs were detected in sediments from the Annick Water. Permethrin was the major contaminant detected in the sediments. The spatial distribution of contaminants in the sediment indicated two sources of contamination on the Annick Water. The spillages and surface water discharges from the wool processing factory and the final effluent discharging from Stewarton STW. The pattern of contaminants detected in the Sewage effluent and the sediments was similar indicating that the STW was the major source of contamination.

The use of sediment quality standards derived by the equilibrium partitioning approach was investigated as a method of assessing the significance of the contaminant concentrations detected in sediments. The lack of reliable partition coefficient data, doubts over the scientific validity of the EPA approach and doubts over the justification of applying toxicity data for pelagic species to benthic species severely limited the use of this technique. At present it is felt that this technique is only suitable as a method of crudely classifying sediments.

Comparison of the concentrations of contaminants detected in the sediments with published sediment contaminant concentrations from other European sites, EPA derived sediment quality standards, and published sediment toxicity data indicated that permethrin was the most significant contaminant detected in the sediments.

Comparison with invertebrate biotic index scores for the Annick Water, fish population studies of the Annick Water, and published sediment toxicity data confirmed that the sediments between the STW and Chapeltoun were acutely toxic to invertebrates.

Invertebrate biotic index scores from the lower reaches of the Annick Water and between the wool processors and the STW were lower than could be explained by comparison with published sediment toxicity data. Faster degradation of trans permethrin in the environment to form a more toxic mixture of permethrin isomers, synergistic activity with organophosphorus pesticides and the negative insecticidal temperature coefficient of permethrin were suggested as possible explanations of these low biotic index scores.

Permethrin was not detected in any of the eels caught from the Annick Water or the Glazert Burn, suggesting that eels can efficiently metabolise permethrin. Dieldrin was the major contaminant detected in eels downstream of the STW. The concentrations of dieldrin detected in these eels were at a level that suggests that regular human consumption of these eels presented a significant hazard to human health. These concentrations are likely to be hazardous to fish eating-birds and aquatic mammals.

The concentrations of HCH isomers, DDT and DDT metabolites detected in eels from the Annick Water and Glazert Burn were below European consumption standards and comparable to published concentrations.

The concentrations of PCBs detected in eels from the Glazert Burn and the Annick Water were in the Medium JMP category, these concentrations are close to those thought to have an effect on the viability of fish eating-bird and aquatic mammal populations.

This study clearly indicates that the discharge of mothproofing chemicals from the wool processing factory and the sewage treatment works at Stewarton is having an adverse effect on the fauna of the Annick Water. On the 1st of January 1995 a new consent was granted for the discharge of sewage effluent from Stewarton STW. This consent requires that the concentration of total permethrin in the effluent discharged to the Annick Water shall not exceed 80ng/l. At present the concentration of permethrin in the effluent from Stewarton STW still frequently marginally exceeds this level. However, the pressure excerted by the application of this consent has resulted in a significant reduction in the amounts of permethrin discharged to the Annick Water during 1995 and 1996. This reduction has resulted in a dramatic improvement on the invertebrate populations of the Annick Water. The most recent national biological classification of river water quality carried out in the summer of 1996 has indicated that the Annick Water upstream of the STW is now of class A and the Annick Water downstream of the STW is of class B (Doughty SEPA pers comm.).

Chapter 5
Mothproofing Chemicals in Irvine Bay.

5.1 Introduction

The largest proportion of inputs from textile and wool processing industry in Scotland is concentrated in four areas: the Tweed, the Annick, Loch Leven and Irvine Bay. Of these, Irvine Bay is the only catchment area where mothproofing chemicals are discharged directly into the marine environment. Chapter 4 investigated in detail the discharge of mothproofing chemicals to the freshwater environment. In this chapter the discharge of these chemicals to a coastal bay, Irvine Bay, is considered.

Ayrshire and Kilmarnock have for many years had a reputation for producing textile and wool products of high quality, the Kilmarnock area in particular has a world wide reputation for the production of high quality wool carpets.

These industries dispose of their trade waste to the local sewerage system which ultimately discharges them into Irvine Bay via two long sea outfalls: The Irvine Valley Sewer (IVS) and the Garnock Valley Sewer (GVS).

The Irvine Valley Sewer is a modern sewerage system which was designed to replace a large number of smaller local sewage treatment works. The sewerage system has a population equivalent of 237,600 and dry weather flow at Meadowhead inlet works of 825 l/sec. The catchment area of IVS is shown in Figure 25 and serves the areas of Darvel, Newmilns, Galston, Hurlford, Kilmarnock, Fenwick, Crosshouse, Springside, Dreghorn, Irvine (most), Dundonald, Symigton, Tarbolton, Barassie, Loans, Troon, Monkton, Prestwick, Ayr (part), Mossblown, Auchincruive, and Kilmaurs.

At the Meadowhead inlet works the effluent receives limited treatment. This treatment comprises of mechanically raked bar screens, grit removal and cleaning by detritors and classifiers, and the interception of solids by drum screens. The screenings are macerated and returned to the flow at the inlet to the plant. Macerated screenings which do not pass through the drum screens are further macerated on subsequent passes. A 2metre diameter gravity sewer 1.7 Km long conveys the treated sewage from Meadowhead to a pumping station at Gailes. Mixed flow pumps at Gailes convey the screened effluent down one of two 2.2Km pipes driven beneath the sea bed.

The Garnock Valley sewer is a modern sewerage system serving the areas of Dalry, Kilwinning, Irvine (part), Stevenston, Adrossan, and Saltcoats. The sewerage system has a population equivalent of 197,676 and a dry weather flow of 475 l/sec at the Stevenston point outfall works. At Stevenston point outfall the effluent receives limited treatment. This treatment comprises of coarse screenings (50 mm and 20 mm screens), detritor grit removal, and fine screening (6 mm screens). The screening are macerated and returned to the effluent flow.

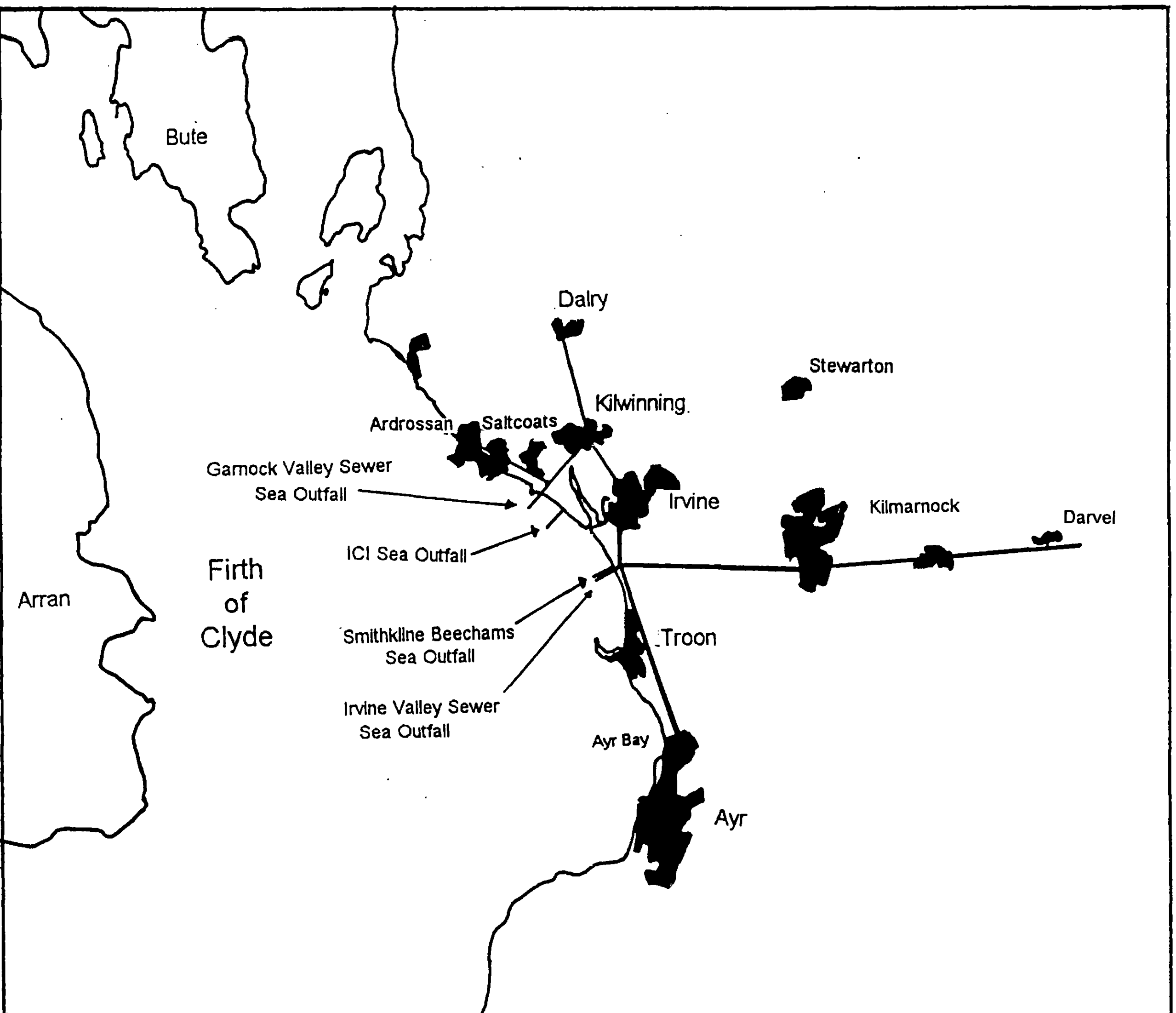
The location and catchment areas of both these sewerage systems along with the other major discharges to Irvine Bay are shown in Figure 25.

A wide variety of industries discharge trade waste to these sewerage systems, examples of the major industries which discharge to the IVS and GVS are listed in Table 115.

Table 115 Examples of Industries which discharge trade effluents to the Irvine and Garnock Valley Sewer Systems.

	Irvine Valley Sewer		Garnock Valley Sewer	
Industry	Company	Effluent Type	Company	Effluent Type
Carpet Manufacture /Wool Processing	Blackwood Brothers Douglas Raeburn	Wool scouring and dyeing effluent Wool scouring and dyeing effluent	None	None
Textile	Strathclyde Knitwear - woollen garment manufacture Moonweave - Lace Manufacture Basford Home Fashions - material finishing	Dyeing washing and finishing effluent Washing and finishing effluent Washing and dyeing effluents	Altmira Colour Laxton Crawford	Dyeing and washing effluent Dyeing and washing effluent
Aviation Industry	British Aerospace, Avial Caledonian	Plating effluent Engine Overhaul Effluent	Tercet Metal Finishers	Plating Effluent
Meat processing	Belcher Foods, Unique Cuisine, Sandyford Foods		Robertson & Son Highland Meats	Abattoir & Equipment Washdown
Food processing	Rowallan Creamery, Ayrshire Food Products	Food manufacture and equipment washdown.	Haynards Foods	Food manufacture and equipment washdown
Electronics Industry	SCI, Prestwick Circuits, Electrconnect	Plating and Finishing effluents	None	None
Paper Industry	Caledonian Paper	general paper making effluent	None	None
Laboratory Effluent	Hannah Institute, SAC West	Laboratory effluent & animal housing washdown	None	None
Pharmaceutical	Smith Kline Beechams	occasional discharges	Roche Products	General mixed effluent from pharmaceutical manufacture
Light Engineering	NACCO - Fork lift truck manufacturer	Pre paint prep & plating effluent	Fullarton Fabrication H.S.P.	Prepaint preparation & plating effluent

Figure 25 Catchment Area of Irvine and Garnock Valley Sewers.



5.2 Location

Irvine Bay lies on the eastern edge of the Firth of Clyde. It is a shallow sandy bay between the Ayrshire towns of Saltcoats and Troon. It is exposed to the prevailing south-west winds and shelter increases from North to South. The southern end is protected by a rocky promontory. The depth gradient reflects this exposure pattern, with the contours being widely spaced in the south end of the bay but very close in the northern part (Eleftheriou *et al.*, 1986). The Rivers Irvine and Garnock meet and discharge to Irvine Bay as a combined system through an estuary mouth which is only about 100 metres wide. The firth itself forms part of a larger estuary system which includes many long lochs with small river inflows (Lewis, 1986).

The water circulation within Irvine Bay is weak, and the direction of water movement is dictated by the wind when it exceeds $7ms^{-1}$; otherwise there is a weak residual movement to the north as part of the general circulation of the firth of Clyde (CRPB, 1985). It has been estimated that the annual mean drift of water through Irvine Bay is north-west at a rate of about 5cm/sec (Lewis, 1986).

The nearshore waters of Irvine Bay are stratified due to the brackish water from the Irvine-Garnock Estuary. Water from the Clyde Estuary overlies denser inshore waters forming an interface at a distance of approx. 3 Km offshore.

Irvine Bay is an important resource and is utilised by the community in a variety of manners as outlined below.

A) Irvine Bay is a key area in the important Clyde Fishery.
It has been identified as the major nursery and feeding ground for young flatfish, notably plaice (*Pleuronectes platessa*) in the Clyde Sea Area (Poxton *et al.*, 1983; Poxton, 1986). A portion of the larval herring (*Clupea harengus*) which hatch in spring on Ballantrae Bank to the South are carried by the current to Irvine Bay, where they may remain to complete their development, or pass through to metamorphose in the inner reaches of the Firth of Clyde. The bay also supports stocks of scampi (*Nephrops norvegicus*) as well as stock of scallops (*Pecten maximus*) and queens (*Chlamys opercularis*). (MacKay *et al.*, 1986)

The bay is of marine biological interest and supports a wide variety of organisms, including rare species such as the polychaete *Commensodorum commensalis*.

B) Nature Reserves
Irvine Bay and the surrounding areas contain a number of nature reserves and Sites of Special Scientific Interest notified by the former Nature Conservancy Council, now Scottish National Heritage (Kerr, 1986), these are listed in Table 116.

Table 116 Nature Reserves and Sites of Special Scientific Interest Within Irvine Bay and Surrounding Areas.

Number	Name / Location	Interest
1	Ardrossan-Saltcoats Coast	Geology: Igneous into coal measures
2	Western Gailes	Plants, invertebrates, dunes.
3	Bogside Flats	Wildfowl, waders, mudflat, saltmarsh
4	Troon Golf links Foreshore	Plants, Birds, Grassland, Dunes
5	Lady Isle	Terns, Seals, Skerry

C) Tourism

Tourism is a very important industry in Ayrshire with a number of caravan parks and yachting / sailing clubs in the area. The beaches of the bay and the surrounding area are of particular importance both as tourist attractions and as a recreation facility for the local population.

D) Industry

Irvine Bay is also heavily utilised for waste disposal, the majority of domestic sewage from the surrounding area is discharged into Irvine Bay via 2 long sea outfall IVS and GVS. In addition there are 2 major industrial outfalls ICI Nobel's Explosives Company, and SmithKline Beechams.

The aim of this study was to investigate the distribution of organochlorine and mothproofing compounds in Irvine Bay following their discharge from IVS and GVS.

5.3 Sampling and Analysis Strategy.

5.3.1 Effluents

To assess the input of the selected target analytes to the bay an initial sampling and analysis of the effluent from the 4 major outfalls (IVS, GVS, ICI, and Beechams) which discharge into Irvine Bay was undertaken. The results (data not presented) show that the target analytes were present only in the effluent from the two valley sewers.

Following these results no further sampling of effluent from ICI and Beechams LSO was undertaken.
Effluent samples from IVS and GVS were collected on a monthly basis between 1993 and 1995. These samples were analysed using method C outlined in section 3.7.3.

5.3.2 Sediments

The collection of sediment samples in the marine environment is very costly and time consuming involving the use of a survey vessel, specialised sampling equipment and occasionally divers. To maximise the use of resources the Irvine Bay sediment samples for this investigation were collected as part of CRPB's routine biological and chemical monitoring program. This is an established program with a network of sites designed to asses the biological and chemical impact of the discharges from IVS, GVS, ICI and Beechams. This sampling strategy allowed the use of existing biological data in the interpretation of the chemical results.

The majority of samples were collected on 28 and 29/7/94 from CRPBs survey vessel the Endrick II using a Day Grab. Separate surface scrapes of these grab samples were taken for particle size, trace metal and organic analysis.

The discharges from Irvine Valley sewer and Smithkline Beechams LSO are located on rocky outcrops. This makes the collection of sediment samples using a Day Grab unpracticable. Three divers were employed by CRPB to collect surface sediments samples from around these outfalls on the 2 and 3/8/94. The samples for organic analysis were collected directly into the sampling jar by the diver on the sea bed.

Graphical representation of the sampling sites is shown in Figures 30, with further detail in Tables 117 and 118. The collection of sediment samples from the Endick II using a Day grab are illustrated in Figures 26-29.

Figure 26 Irvine Bay Sediment Collection: Day Grab Being Released from Endrick II.

Figure 27 Irvine Bay Sediment Collection: Day Grab Being Lowered to Seabed.

Figure 28 Irvine Bay Sediment Collection: Day Grab Being Brought Back on Board Endrick II.

Figure 29 Irvine Bay Sediment Collection: Day Grab Being Opened to Obtain Surface Sediment.

Figure 30 Irvine Bay Sampling Sites.

Day Grab Survey

Ardrossan
GVS Sea Outfall
ICI Sea Outfall
Irvine
IVS Sea Outfall
Diving Survey Sites
Troon
J. J1. L8 L81 L6 L7.
I. H. F. Z. E. C. P. Q2. Q1. Q. R1.
2 km

Diving Survey

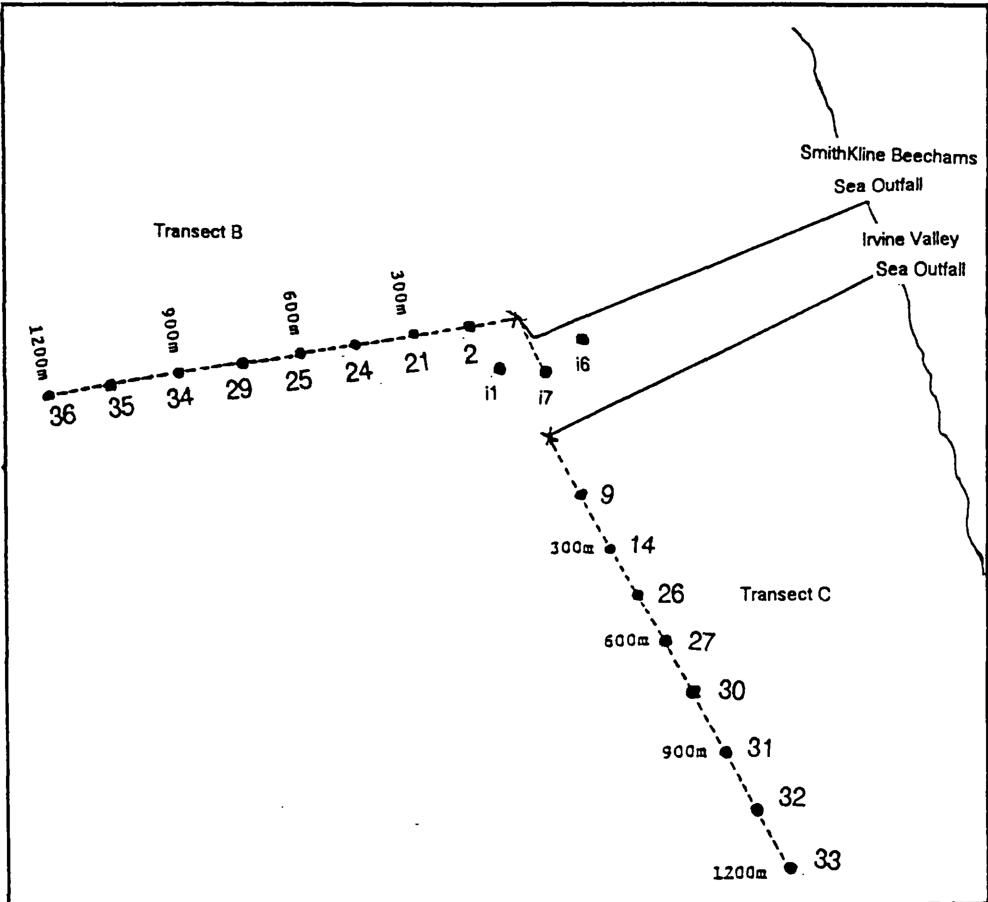


Table 117 Irvine Bay Day Grab Survey Sampling Locations

Site No.	Latitude	Longitude	Sediment Type	Depth m
J	55 37.25 N	4 45.75 W	SAND	14
I	55 36.72 N	4 46.55 W	SANDY MUD	29
H	55 35.92 N	4 47.40 W	MUD	38
F	55 35.15 N	4 48.25 W	MUD	52
J1	55 37.02 N	4 45.38 W	SANDY MUD	17
L8	55 36.95 N	4 45.35 W	MUD	17
L7	55 36.85 N	4 45.45 W	SAND	21
L81	55 36.92 N	4 45.18 W	SANDY MUD	17
L6	55 36.90 N	4 45.00 W	SANDY MUD	17
Q2	55 35.92 N	4 44.15 W	SAND	20
Q1	55 35.72 N	4 43.80 W	SANDY MUD	20
Q	55 35.78 N	4 43.25 W	SAND	20
P	55 35.30N	4 44.45 W	COARSE SAND	25
Z	55 34.75 N	4 45.20 W	MUD	40
E	55 33.60 N	4 45.60 W	MUD	53
R1	55 34.05 N	4 40.55 W	COARSE SAND	9
C	55 33.60 N	4 43.95 W	MUD	36
AB1	55 27.88 N	4 40.00 W	COARSE SAND	10
AB3	55 29.32 N	4 42.45 W	COARSE GRIT	13

Table 118 Irvine Bay Diving Survey Sampling Locations

Site No.	Latitude	Longitude	Sediment Type	Depth m
B2	55 35.05 N	4 41.53 W	HEAVY FLOCCULENT	10
B21	55 34.99 N	4 41.60 W	HEAVY FLOCCULENT	10
B24	55 34.92 N	4 41.75 W	GRAVEL, MEDIUM FLOCCULENT	11
B25	55 34.87 N	4 41.80 W	COARSE SAND, LIGHT FLOCCULENT	11
B29	55 34.82 N	4 41.95 W	GRAVEL, MEDIUM FLOCCULENT	11
B34	55 34.78 N	4 42.00 W	GRAVEL, MEDIUM FLOCCULENT	12
B35	55 34.70 N	4 42.15 W	ROCKS, MEDIUM FLOCCULENT	15
B36	55 34.65 N	4 42.25 W	ROCKS, MEDIUM FLOCCULENT	15
C9	55 34.90 N	4 41.32 W	GRAVEL, VERY HEAVY FLOCCULENT	10
C14	55 34.84 N	4 41.25 W	GRAVEL, VERY HEAVY FLOCCULENT	10
C26	55 34.78 N	4 41.18 W	GRAVEL, VERY HEAVY FLOCCULENT	8
C27	55 34.71 N	4 41.10 W	COARSE GRAVEL, VERY HEAVY FLOCCULENT	8
C31	55 34.58 N	4 40.90 W	COARSE GRAVEL, VERY HEAVY FLOCCULENT	8
C32	55 34.50 N	4 40.80 W	COARSE GRAVEL, VERY HEAVY FLOCCULENT	8
C33	55 34.45 N	4 40.70 W	COARSE GRAVEL, VERY HEAVY FLOCCULENT	7
I1	55 35.04 N	4 41.48 W	COARSE GRAVEL, MEDIUM FLOCCULENT	10
I6	55 35.14 N	4 41.30 W	COARSE GRAVEL, VERY HEAVY FLOCCULENT	10
I7	55 35.06 N	4 41.30 W	COARSE GRAVEL, VERY HEAVY FLOCCULENT	10

5.4 Results

5.4.1 Effluent Samples

Between January 1993 and December 1995, 32 and 36 twenty-four hour composite samples of the effluent from Irvine and Garnock Valley sewers were collected and analysed for the 28 organochlorine compounds listed in Table3.

19 of the target compounds including all the PCBs, the industrial solvents HCBD, HCB and the insecticides endosulfan, aldrin and endrin were not detected in the effluents sampled.
The 9 compounds most frequently detected in the effluents were α HCH, β HCH, γ HCH, pp DDE, pp DDD, pp DDT, dieldrin, cis and trans permethrin.

The results of the analysis for the compounds which were frequently detected are summarised in Tables119-120.

Table 119 Compounds frequently detected in 24 hour composite samples of effluent from Irvine Valley Sewer 1993-1995.

Compound	No. of observations	% of results in which compound was positively detected	Mean concentration of positive results ng/l	Median concentratio of positive results ng/l	Maximum concentration ng/l
cis Permethrin	32	84	381	328	1256
trans Permethrin	32	84	594	496	2014
Dieldrin	32	75	23.9	19	81.9
pp DDE	32	47	4.6	3.6	13.3
pp DDD	32	16	4.4	4.9	7.3
pp DDT	32	53	16.1	9.8	54
α HCH	32	44	3.4	3.3	6.4
β HCH	32	47	10.7	9.8	25
γ HCH	32	94	37.9	31.6	106

Table 120 Compounds frequently detected in 24 Hour Composite Samples of Effluent From Garnock Valley Sewer 1993-1995.

Compound	No. of observations	% of results in which compound was positively detected	Mean concentration of positive results ng/l	Median concentratio of positive results ng/l	Maximum concentration ng/l
cis Permethrin	36	69	398.4	255	1985
trans Permethrin	36	72	714.8	530	2751
Dieldrin	36	56	7.7	7.4	15.3
pp DDE	36	11	3.2	3.5	5.2
pp DDD	36	22	3.2	3.2	7.45
pp DDT	36	33	21.6	10.9	74
α HCH	36	58	7.5	3.3	51.9
β HCH	36	39	11.3	7.3	38.4
γ HCH	36	81	33.2	28.5	100

The results obtained from the analysis of the effluent from IVS and GVS are similar to the results obtained from the analysis of effluent from Stewarton STW.

The compound detected most frequently in effluent from IVS and GVS was lindane. Lindane was detected in 81 and 94 % of samples analysed. Effluent concentrations ranged from <1 - 106 ng/l and <1 - 100 ng/l in IVS and GVS respectively. Typical concentrations for the effluents were 30 ng/l. The isomers of Lindane α HCH and β HCH were detected much less frequently and at much lower concentrations than lindane in both effluents. These results are similar to those from Stewarton STW in that the concentrations of α HCH and β HCH are close to the detection limit of the analytical method.

Permethrin was also frequently detected (84 % and 72 % IVS and GVS respectively) in the effluent. Permethrin was present in the effluent at much higher concentrations than any of the other organochlorine contaminants. Total Permethrin concentrations in the effluent ranged from <20 - 3270 and <20 - 4736 ng/l in IVS and GVS respectively.

Dieldrin was also detected in most (75 % and 56 % IVS and GVS respectively) of the samples with typical concentrations in the effluent from IVS and GVS of 19 and 7 ng/l respectively.
PP DDT and its metabolites pp DDD and pp DDE were detected in both effluents. PP DDT was the most frequently detected member of this group of compounds both in IVS and GVS. Typical concentrations in both effluents were 10 ng/l. pp DDE was also frequently detected in IVS, typical concentrations were approximately 4 ng/l. pp DDE was only detected occasionally in effluent from GVS, typical concentrations were also 4 ng/l. pp DDD was detected only occasionally in effluent from both IVS and GVS, typical concentrations were approximately 4 ng/l in both effluents.

5.5.2 Sediment Samples

5.5.2.1 Total Organic Carbon and Nitrogen

The results for total organic carbon (TOC), nitrogen and particle size distribution in the sediments from Irvine Bay are listed in Tables 120 to 121.

Table 120. Percentage Total Organic Carbon and Nitrogen in Sediments from Irvine Bay.

Site No.	% Total Organic Carbon	% Nitrogen	C/N ratio
J	1.38	0.08	16.5
I	1.92	0.11	17.3
H	1.68	0.01	151
F	2.33	0.16	14.4
J1	1.40	0.09	16.3
L8	1.97	0.21	9.32
L7	1.12	0.07	15.7
L81	1.75	0.10	17.3
L6	0.64	0.02	39.9
Q2	0.61	0.06	10.8
Q1	0.94	0.03	27.6
Q	0.57	0.02	22.6
P	1.47	0.04	40.8
Z	1.23	0.11	11.5
E	1.74	0.22	7.89
R1	0.45	0.06	7.14
C	0.67	0.21	2.92
AB1	0.60	0.01	67.0
AB3	0.34	0.05	6.45
B2	2.42	0.19	12.8
B21	3.18	0.27	11.9
B24	1.14	0.12	9.33
B25	0.65	0.06	10.8
B29	0.47	0.13	3.56
B34	0.65	0.06	11.9
B39	0.75	0.08	9.34
B36	0.84	0.07	12.1
C9	2.56	0.21	12.1
C14	0.85	0.23	3.68
C26	0.86	0.08	11.0
C27	0.99	0.27	3.69
C31	2.93	0.12	25.2
C32	1.21	0.23	5.23
C33	1.34	0.08	17.6
I1	1.18	0.09	12.6
I6	0.81	0.08	10.0
I7	0.79	0.07	11.3

Table 121. Particle Size Distribution in Sediments from Irvine Bay

		% of Total Weight								
Site No.	modal particle size <500μm	0-11.55 μm	11.55 - 25.46 μm	25.46 - 56.09 μm	56.09 - 101.44 μm	101.44 - 492.47 μm	0 - 0.5 mm	0.5 - 1 mm	1 - 2 mm	>2 mm
J	239.23	4.63	2.76	0.24	19.28	71.45	98.36	0.35	0.34	0.96
I	178.45	21.75	8.49	8.29	26.63	24.59	89.75	5.52	3.79	0.94
H	90.75	31.54	10.60	14.14	17.89	15.50	89.67	8.79	1.54	0
F	7.92	43.09	14.27	12.79	5.44	4.96	80.55	16.97	2.4	0.07
J1	129.65	3.44	1.82	2.48	5.84	11.07	24.64	31.72	18.26	25.38
L8	192.17	16.53	9.15	10.76	26.72	25.25	88.42	4.96	3.68	2.95
L7	115.69	12.73	6.02	6.04	36.02	33.78	94.59	2.45	2.16	0.81
L81	119.77	17.72	9.72	8.75	26.17	31.76	94.12	1.28	0.6	3.99
L6	163.39	9.95	5.28	2.60	19.44	60.95	98.23	0.68	0.61	0.48
Q2	172.22	13.01	6.05	3.48	15.09	59.20	96.83	2.03	0.79	0.35
Q1	221.01	9.20	4.08	2.99	8.63	68.83	93.73	2.07	1.06	3.13
Q	198.91	6.20	3.23	1.07	10.22	77.66	98.38	1.59	0.35	0.12
P	195.54	10.21	4.25	2.11	12.01	68.41	96.99	1.76	.87	0.39
Z	81.93	32.01	11.75	19.29	23.14	6.44	92.63	5.97	1.35	0.06
E	8.22	42.89	15.07	14.47	5.66	2.13	80.22	17.40	1.71	0.66
R1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C	121.85	17.27	6.43	6.03	24.45	30.58	84.76	6.46	6.36	2.42
AB1	160.35	5.87	3.26	0.50	16.53	69.12	95.27	3.33	0.74	0.66
AB3	259.88	7.23	2.86	2.61	5.52	51.67	69.89	7.64	8.12	14.35
B2	133.57	3.18	2.44	2.86	4.92	10.71	24.11	7.28	14.49	54.12
B21	117.16	17.95	13.15	13.95	20.25	27.59	92.89	2.79	3.02	1.31
B24	119.85	1.18	0.79	1.18	1.66	4.33	9.15	10.19	17.14	63.52
B25	336.85	6.57	3.11	3.26	4.94	36.80	54.67	8.71	6.11	30.51
B29	136.78	3.90	2.12	1.93	6.49	16.92	31.36	8.35	9.81	50.47
B34	366.63	7.59	3.46	3.35	5.30	19.38	39.09	10.14	9.26	41.51
B35	127.11	ND	ND	ND	ND	ND	ND	ND	ND	ND
B36	108.81	11.72	5.54	4.84	4.77	10.74	37.61	14.19	17.09	31.2
C9	143.34	0.27	0.21	0.27	0.80	2.10	3.65	1.45	3.09	91.81
C14	119.85	0.59	0.44	0.57	0.73	4.57	6.91	6.19	6.65	80.25
C26	141.95	2.69	1.70	1.83	4.01	15.43	25.66	10.67	13.08	50.58
C27	139.42	2.41	1.78	1.93	1.67	4.09	11.89	5.97	10.03	72.11
C31	416.23	3.03	2.21	2.58	3.36	15.20	26.38	23.64	17.58	32.4
C32	402.64	ND	ND	ND	ND	ND	ND	ND	ND	ND
C33	124.64	2.09	1.13	1.03	2.41	3.82	10.49	5.69	7.62	76.19
I1	143.03	2.32	1.68	1.44	8.62	21.52	35.57	2.24	6.46	56.14
I6	161.52	1.32	0.85	0.75	2.41	9.24	14.57	3.07	6.54	75.82
I7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND - No data presented

The spatial distribution of % TOC and modal particle size are presented visually in Figures 31-34.

The sediment samples from Irvine Bay were generally of uniform composition consisting of well sorted sand and mud dominated by particles of less than 0.5 mm particle size. Laser granulaometer analysis of the less than 0.5 mm particles indicated that there is a clear particle size gradient with increasing depth.

5.4.2.2 Organochlorine Compounds

Results for organochlorine compounds in sediment samples from Irvine Bay are presented in Tables 122 and 123.

These results are also shown in figures 35-40.

Detectable concentrations of organochlorine compounds were present in all the sediment samples examined from Irvine Bay.

The pattern of contamination observed in sediments from Irvine Bay is similar to the pattern observed in the sediments from the Annick Water. Organochlorine insecticide metabolites and organochlorine insecticides detected in sediments from the Annick Water were also detected in sediments from Irvine Bay. As per sediments from the Annick Water polychlorinated biphenyls were also detected in all of the sediments examined.

To allow comparison with published data chlorobiphenyl concentrations have been expressed as the sum of the seven congeners specified by the International Council for the Exploration of the Seas (ICES).

Figure 31 Irvine Bay Diving Survey: Organic Carbon in Surface Sediment (%)

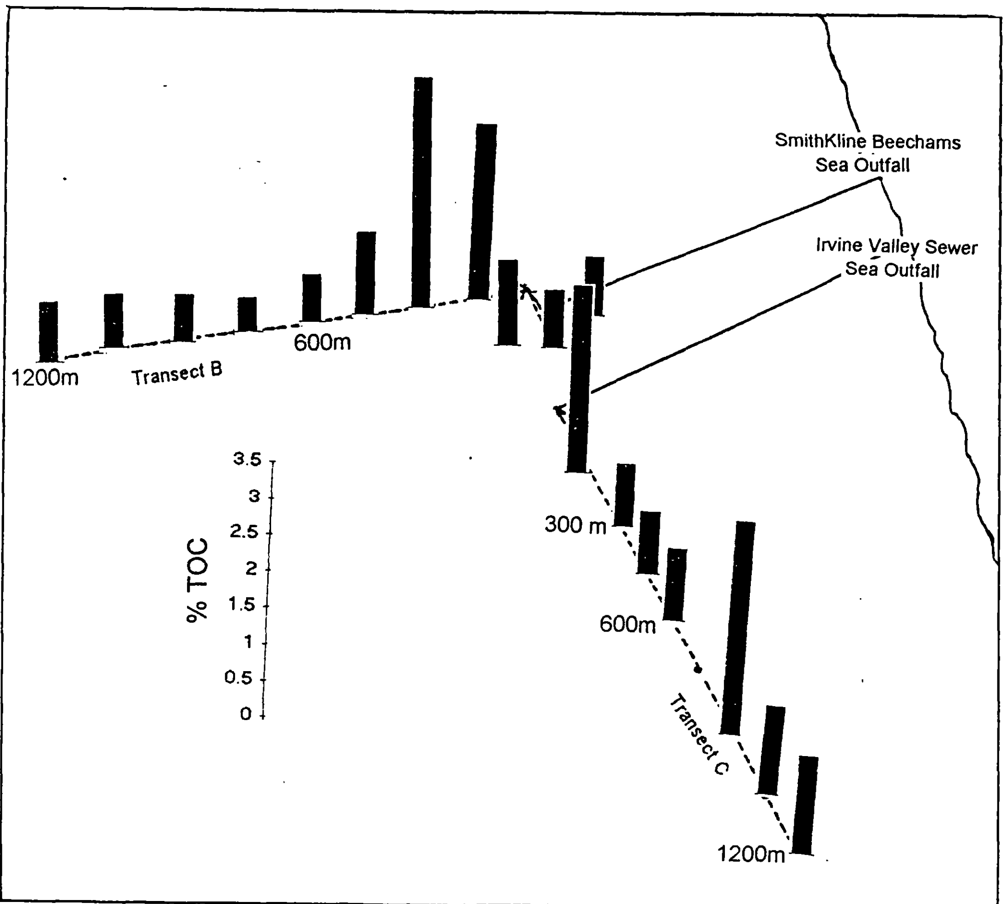


Figure 32 Irvine Bay Grab Survey: Organic carbon in Surface Sediment (%)

GVS Sea Outfall
ICI Sea Outfall
IVS
Sea Outfall
Diving
Survey Sites
% TOC
3.5
3
2.5
2
1.5
1
0.5
0
2 km

Figure 33 Irvine Bay Diving Survey: Modal Partilce Size (μm).

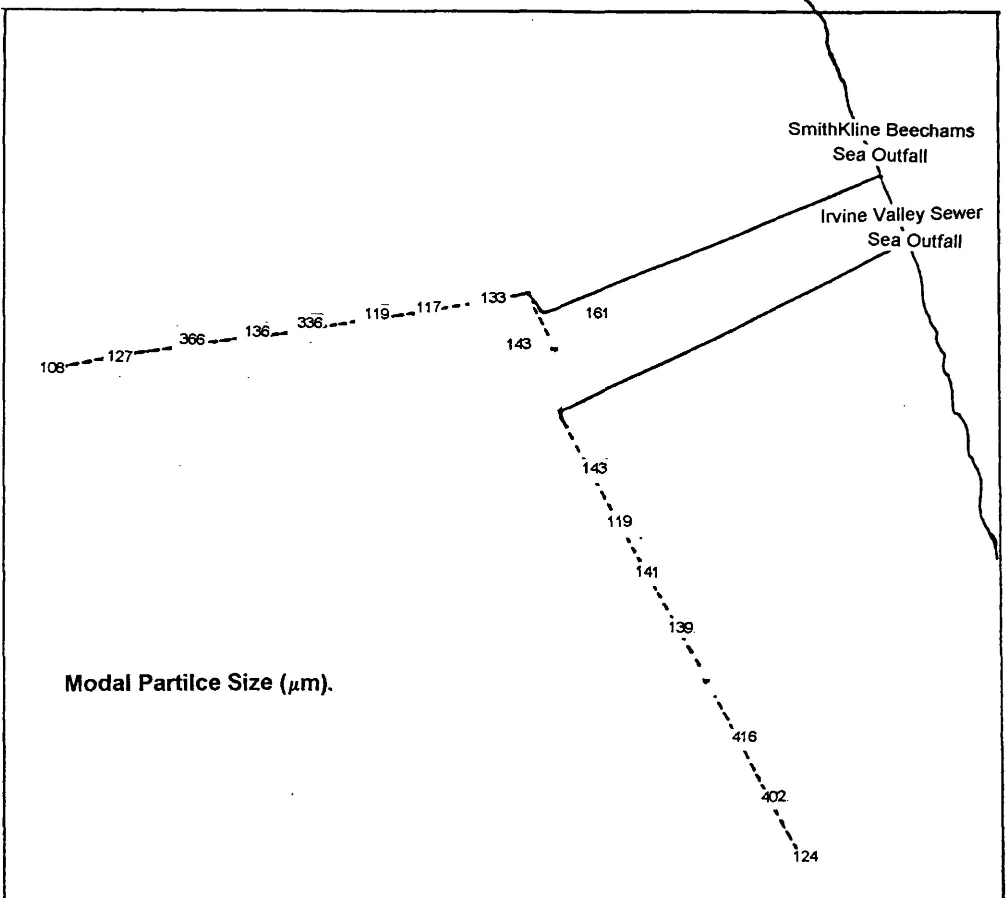


Figure 34 Irvine Bay Grab Survey: Modal Particle Size (μm).

GVS Sea Outfall
ICI Sea Outfall
IVS
Sea Outfall
Diving
Survey Sites
239
129
192
115
163
178
172
221
198
90
195
7
81
8
2 Km

Table 122 Concentrations of Organochlorine Pesticides in Sediments from Irvine Bay and Surrounding Area (ng/g dry Weight)

Site No.	Cis Permethrin	Trans Permethrin	Total Permethrin	Dieldrin	PP DDT	PP DDD	PP DDE	α HCH	β HCH	γ HCH
J	<0.5	<0.5	<1.0	<0.1	<0.2	<0.2	<0.2	<0.1	NA	<0.1
I	2.15	3.83	5.98	0.32	0.4	0.42	0.49	0.13	NA	2.42
H	1.33	1.15	2.48	0.35	0.7	1.38	1.05	<0.1	1.44	0.88
F	4.12	5.29	9.41	0.56	0.5	1.06	1.08	<0.1	NA	0.86
J1	3.4	4.37	7.77	0.46	0.4	0.87	0.39	0.28	NA	2.99
L8	6.51	10.55	17.06	2.49	1.2	<0.20	0.56	0.82	NA	5.95
L7	<1.0	<1.0	<2.0	0.21	<0.2	0.61	0.46	<0.1	1.1	1.28
L81	6.5	10.12	16.62	0.46	1.6	0.77	0.77	0.65	NA	4.18
L6	2	2.24	4.24	0.22	0.8	0.43	0.39	<0.1	0.95	1.25
Q2	<0.5	<0.5	<1.0	0.32	<0.2	<0.2	0.2	<0.1	0.66	0.81
Q1	<0.5	<0.5	<1.0	<0.1	<0.2	<0.2	0.24	<0.1	0.8	0.44
Q	<0.5	<0.5	<1.0	<0.1	<0.2	<0.2	0.24	<0.1	0.41	0.57
P	<0.5	<0.5	<1.0	<0.1	<0.2	<0.2	<0.2	<0.1	0.65	0.63
Z	<0.5	<0.5	<1.0	0.4	<0.2	<0.2	0.98	<0.1	1.67	0.79
E	1.06	0.97	2.03	0.66	0.44	2.3	1.18	0.12	NA	1.04
R1	<0.5	<0.5	<1.0	0.24	1.46	0.73	0.29	<0.1	NA	1.1
C	<0.5	<0.5	<1.0	<0.1	0.45	0.33	0.44	<0.1	1.21	0.77
AB1	<0.5	<0.5	<1.0	<0.1	<0.2	<0.2	<0.2	<0.1	NA	<0.1
AB3	<0.5	<0.5	<1.0	<0.1	<0.2	<0.2	<0.2	<0.1	NA	<0.1
B2	28.1	27.7	55.9	1.77	2.1	1.57	0.82	2.43	NA	39.47
B21	99.0	65.7	165	3.37	2.21	2.85	1.44	14.7	NA	136.31
B24	12.8	11.1	23.9	1.58	0.86	1.58	0.92	3.88	NA	21.78
B25	1.73	1.06	2.79	1.42	0.54	0.35	0.31	0.17	NA	1.13
B29	<0.5	<0.5	<1.0	0.55	<0.2	0.81	0.75	<0.1	NA	1.50
B34	2.50	4.95	7.45	0.25	0.32	0.27	0.7	0.16	NA	1.38
B35	1.71	2.66	4.37	0.29	<0.2	0.38	0.39	0.38	NA	1.29
B36	<0.5	<0.5	1.0	<0.1	<0.2	1.01	0.53	<0.1	NA	0.41
C9	42.3	42.0	84.3	2.48	0.91	1.74	1.13	6.94	NA	43.33
C14	7.20	6.09	13.29	0.67	0.76	0.69	0.45	1.26	NA	8.5
C26	2.89	4.95	7.81	0.36	0.41	0.32	0.32	0.29	NA	1.8
C27	10.9	8.55	19.5	0.88	0.48	0.93	0.67	3.11	NA	2.88
C31	4.30	2.92	7.23	0.66	0.53	0.61	0.34	0.39	NA	2.44
C32	2.40	1.79	4.20	0.45	0.54	0.42	0.42	0.12	NA	1.07
C33	10.7	6.87	17.6	1.1	0.82	1.59	2.31	032	NA	3.03
I1	3.43	3.81	7.25	2.99	0.95	<0.2	<0.2	0.27	NA	1.56
I6	1.84	2.42	4.30	2.57	0.61	<0.2	<0.2	0.14	NA	1.41
I7	3.75	3.42	7.18	0.32	0.64	0.53	0.41	0.21	NA	1.75

NA results not available due to presence of coextracted interfering compound in sample extracts.

Table 123 Concentrations of Polychlorinated Biphenyls in Sediments from Irvine Bay (ng/g dry weight)

Site No.	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 105	PCB 138	PCB 156	PCB 180	Sum +ve ICES 7
J	0.39	0.14	0.06	<0.05	0.11	<0.05	<0.05	<0.05	<0.05	0.72
I	0.46	0.20	0.18	0.19	0.40	0.08	0.26	<0.05	0.16	1.86
H	0.84	0.46	0.32	0.40	0.63	0.17	0.51	0.05	0.33	3.49
F	0.66	0.28	0.34	0.41	0.78	0.21	0.57	0.06	0.35	3.39
J1	0.44	0.18	0.12	0.09	0.22	<0.05	0.20	<0.05	0.08	1.33
L8	0.53	0.34	0.28	0.22	0.48	0.09	0.34	<0.05	0.28	2.46
L7	0.67	0.39	0.22	0.22	0.25	0.08	0.24	<0.05	0.12	2.10
L81	0.87	0.50	0.64	0.55	0.83	0.25	0.58	0.08	1.01	4.97
L6	0.69	0.39	0.22	0.18	0.18	0.20	0.20	<0.05	0.07	1.93
Q2	0.59	0.23	0.15	0.16	0.16	<0.05	0.16	<0.05	0.06	1.51
Q1	0.74	0.33	0.16	0.17	0.17	<0.05	0.19	<0.05	0.06	1.82
Q	0.63	0.24	0.13	0.14	0.13	0.05	0.14	<0.05	0.04	1.46
P	0.64	0.29	0.18	0.17	0.17	0.06	0.18	<0.05	0.06	1.70
Z	0.73	0.45	0.37	0.43	0.66	0.18	0.52	<0.05	0.30	3.45
E	0.57	0.31	0.41	0.48	0.83	0.24	0.64	0.08	0.39	3.64
R1	0.57	0.20	0.12	0.12	0.29	0.06	0.17	<0.05	0.09	1.56
C	0.74	0.39	0.21	0.23	0.30	0.09	0.27	<0.05	0.11	2.24
AB1	0.31	0.13	0.05	0.04	0.12	<0.05	0.05	<0.05	0.06	0.76
AB3	0.34	0.12	0.07	0.06	0.14	<0.05	0.08	<0.05	0.05	0.86
B2	<5.69	<1.42	<0.49	0.33	0.61	0.44	0.41	<0.05	0.56	1.91
B21	0.66	0.29	0.80	0.69	0.74	0.35	0.69	0.19	0.82	4.69
B24	<3.47	<0.86	0.26	0.35	0.25	0.25	0.40	<0.05	0.60	1.86
B25	<4.26	<0.95	<0.20	0.34	0.45	<0.33	0.20	<0.05	0.19	1.18
B29	<2.77	<0.69	0.15	<0.11	0.12	0.16	0.11	<0.05	0.13	0.51
B34	<4.12	0.17	0.38	0.24	0.24	0.12	0.24	<0.05	0.05	1.32
B35	<4.35	<0.97	0.71	<0.38	0.41	<0.54	<0.51	<0.05	0.20	1.32
B36	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	ND
C9	<6.83	<1.70	<0.51	<0.26	0.19	0.50	0.24	<0.05	0.46	0.89
C14	<0.05	<1.71	<0.51	<0.18	<0.49	0.47	<0.05	<0.05	<0.22	ND
C26	<4.68	<1.05	<0.58	0.35	0.46	<0.05	0.22	<0.05	0.21	1.24
C27	<3.05	<0.76	<0.23	0.22	0.11	0.25	0.10	<0.05	0.16	0.58
C31	<2.27	<0.56	<0.17	<0.08	0.06	<0.07	0.06	<0.05	0.07	0.19
C32	<3.67	<0.82	0.15	<0.43	0.37	0.18	<0.20	<0.05	0.17	0.54
C33	<6.79	1.07	<0.50	<0.18	<0.51	0.58	0.31	<0.05	0.44	1.82
I1	<4.79	<1.07	<0.60	0.39	0.45	0.91	0.22	<0.05	0.22	1.28
I6	<4.41	<1.06	<0.23	0.47	0.35	0.22	0.21	0.20	0.20	1.23
I7	<5.06	<1.13	0.29	0.59	0.44	<0.42	0.24	<0.05	0.23	1.79

ND Not detected

Figure 35 Irvine Bay Total Permethrin at Selected Sites (ng/g Dry Weight).

Figure 36 Irvine Bay Dieldrin at Selected Sites (ng/g Dry Weight).

Figure 37 Irvine Bay PP DDT at Selected Sites (ng/g Dry Weight).

Figure 38 Irvine Bay PP DDE at Selected Sites (ng/g Dry Weight).

Figure 39 Irvine Bay γ HCH at Selected Sites (ng/g Dry Weight).

Figure 40 Irvine Bay Total PCBs at Selected Sites (ng/g Dry Weight).

5.5 Discussion

5.5.1 Effluent Data

The results from the analysis of the effluent from Irvine and Garnock Valley sewers are similar to those obtained from the analysis of effluent from Stewarton STW.

The nine insecticides (α, β, and γ HCH, pp DDT, pp DDD, pp DDE, dieldrin, cis and trans Permethrin) detected in effluent from Stewarton STW were also detected in the effluent Irvine and Garnock Valley Sewers effluent.

The concentrations and pattern of these contaminants is similar in both valley sewers and Stewarton STW. This suggests a common source of these chemicals and this is likely to be wool treatment processes. As previously observed at Stewarton STW it is likely that Dieldrin, HCH isomers, and DDT arise mainly from the treatment of imported fleeces.

5.5.2 Sediment Data

5.5.2.1 Particle Size analysis, % TOC, % Nitrogen

The results obtained for particle size analysis, organic carbon and nitrogen are in good agreement with those of previous published studies (Eleftheriou *et al*., 1986) from Irvine Bay and the Firth of Clyde.

These results indicate that the sediments from Irvine bay consist mainly of sand and mud. Most of the samples are dominated by particles smaller than 0.5 mm. Examination of the modal less than 0.5mm particle size indicates a particle size gradient within the bay. Deep offshore sites have a higher abundance of smaller particles than shallower inshore sites.

5.5.2.2 Organochlorine Compounds

In general the concentrations of organochlorine compounds in sediments from Irvine Bay are low. The highest concentrations of organochlorine compounds were detected at the sites close to the outfalls (B21, B2, C9, C34, and L8) and at sites with a high concentrations of organic carbon (E and F). As discussed in section 4.6.3 a variety of factors influence sediment adsorption of contaminants. To minimise the influence of these effects and allow analysis of the spatial distribution of the contaminants they have been normalised to 1 % organic carbon. These organic carbon normalised concentrations are listed in Table 124.

Table 124 Concentrations of Organochlorine Compounds in Sediments from Irvine Bay Normalised to 1 % TOC.

Site No.	Total Permethrin	Dieldrin	PP DDT	PP DDD	PP DDE	α HCH	β HCH	γ HCH	Sum ICES 7 PCBs
J	<0.72	<0.07	<0.14	<0.14	<0.14	<0.07	ND	<0.07	0.52
I	3.11	0.17	0.21	0.22	0.25	0.07	ND	1.26	0.97
H	1.48	0.21	0.42	0.82	0.63	<0.06	0.86	0.53	2.08
F	4.04	0.24	0.21	0.46	0.46	<0.04	ND	0.37	1.46
J1	5.53	0.33	0.28	0.62	0.28	0.20	ND	2.13	0.95
L8	8.67	1.27	0.61	<0.10	0.28	0.42	ND	3.02	1.25
L7	<1.79	0.19	<0.18	0.55	0.41	<0.09	0.99	1.15	1.88
L81	9.49	0.26	0.91	0.44	0.44	0.37	ND	2.39	2.84
L6	6.65	0.34	1.25	0.67	0.61	<0.16	1.49	1.96	3.03
Q2	<1.65	0.53	<0.33	<0.33	0.33	<0.16	1.09	1.33	2.49
Q1	<1.06	<0.11	<0.21	<0.21	0.26	<0.11	0.85	0.47	1.94
Q	<1.77	<0.18	<0.35	<0.35	0.42	<0.18	0.72	1.01	2.58
P	<0.68	<0.07	<0.14	<0.14	<0.14	<0.07	0.44	0.43	1.16
Z	<0.81	0.33	<0.16	<0.16	0.80	<0.08	1.36	0.64	2.81
E	1.17	0.38	0.25	1.32	0.68	0.07	ND	0.60	2.10
R1	<2.22	0.53	3.24	1.62	0.64	<0.22	ND	2.44	3.47
C	<1.62	<0.16	0.73	0.54	0.71	<0.16	1.96	1.25	3.64
AB1	<1.66	<0.17	<0.33	<0.33	<0.33	<0.17	ND	<0.17	1.26
AB3	<2.92	<0.29	<0.58	<0.58	<0.58	<0.29	ND	<0.29	2.51
B2	23.05	0.73	0.87	0.65	0.34	1.00	ND	16.28	0.79
B21	51.85	1.06	0.69	0.90	0.45	4.62	ND	42.84	1.47
B24	20.98	1.39	0.76	1.39	0.81	3.41	ND	19.12	1.63
B25	4.31	2.19	0.83	0.54	0.48	0.26	ND	1.74	1.82
B29	<2.11	1.16	<0.42	1.71	1.59	<0.21	ND	3.17	1.08
B34	11.39	0.38	0.49	0.41	1.07	0.24	ND	2.11	2.02
B35	5.85	0.39	<0.27	0.51	0.52	0.51	ND	1.73	1.77
B36	1.20	<0.12	<0.24	1.21	0.63	<0.12	ND	0.49	ND
C9	32.90	0.97	0.36	0.68	0.44	2.71	ND	16.91	0.35
C14	15.58	0.79	0.89	0.81	0.53	1.48	ND	9.96	ND
C26	9.11	0.42	0.48	0.37	0.37	0.34	ND	2.10	1.45
C27	19.68	0.89	0.48	0.94	0.68	3.14	ND	2.91	0.59
C31	2.46	0.22	0.18	0.21	0.12	0.13	ND	0.83	0.06
C32	3.47	0.37	0.45	0.35	0.35	0.10	ND	0.89	0.45
C33	13.14	0.82	0.61	1.19	1.73	23.90	ND	2.26	1.36
I1	6.16	2.54	0.81	<0.17	<0.17	0.23	ND	1.33	1.09
I6	5.29	3.16	0.75	<0.25	<0.25	0.17	ND	1.73	1.51
I7	9.08	0.40	0.81	0.67	0.52	0.27	ND	2.21	2.26

These results are also illustrated in Figures 42-52.

Figure 42 Irvine Bay Diving Survey: Total Permethrin Normalised to 1% TOC (ng/g Dry Weight).

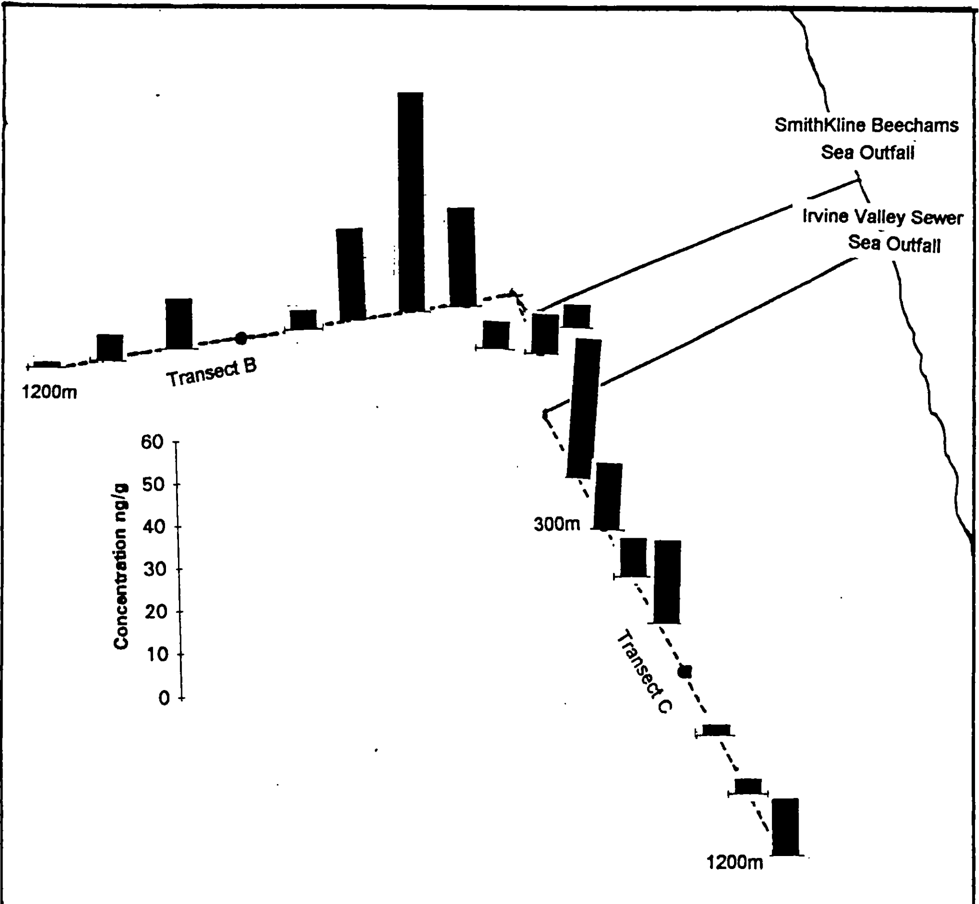


Figure 43 Irvine Bay Grab Survey: Total Permethrin Normalised to 1% TOC (ng/g Dry Weight)

GVS Sea Outfall
ICI Sea Outfall
IVS
Sea Outfall
Diving
Survey Sites
Concentration ng/g
60
50
40
30
20
10
0
2 km

Figure 44 Irvine Bay Diving Survey: Dieldrin Normalised to 1% TOC (ng/g Dry Weight).

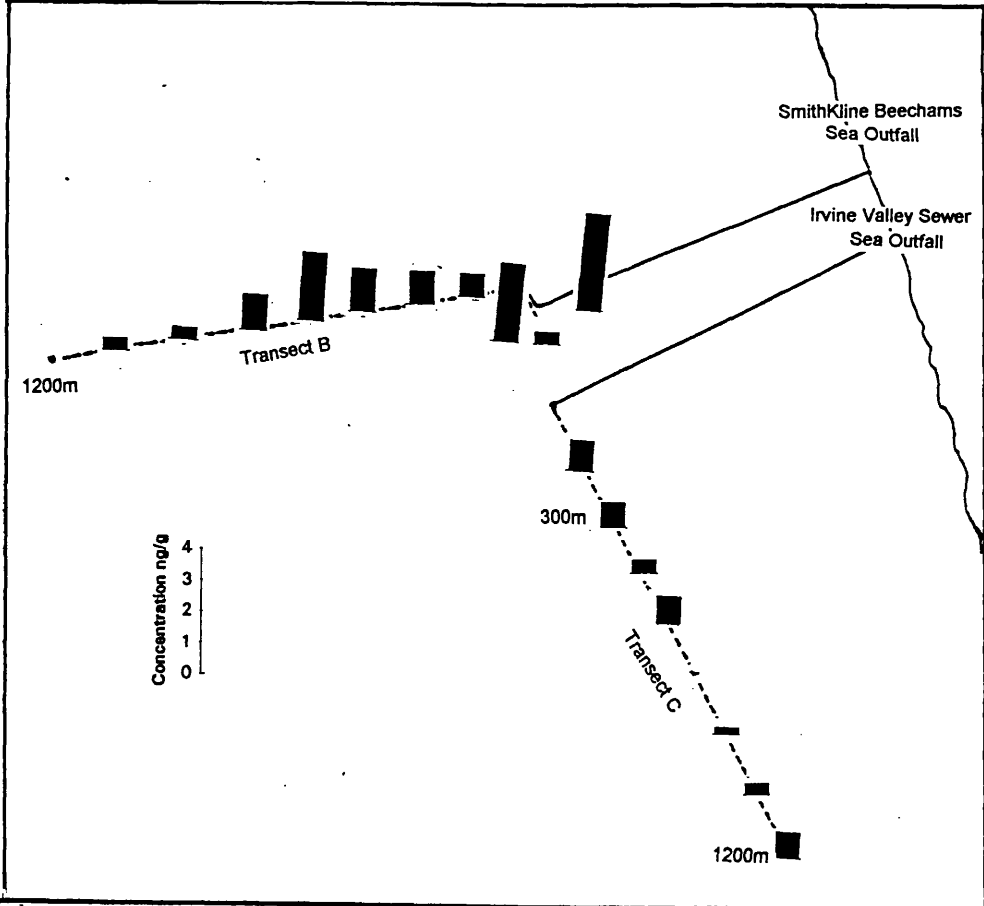


Figure 45 Irvine Bay Day Grab Survey: PP DDT Normalised to 1% TOC (ng/g Dry Weight).

Figure 46 Irvine Bay Diving Survey: PP DDT Normalised to 1% TOC (ng/g Dry Weight).

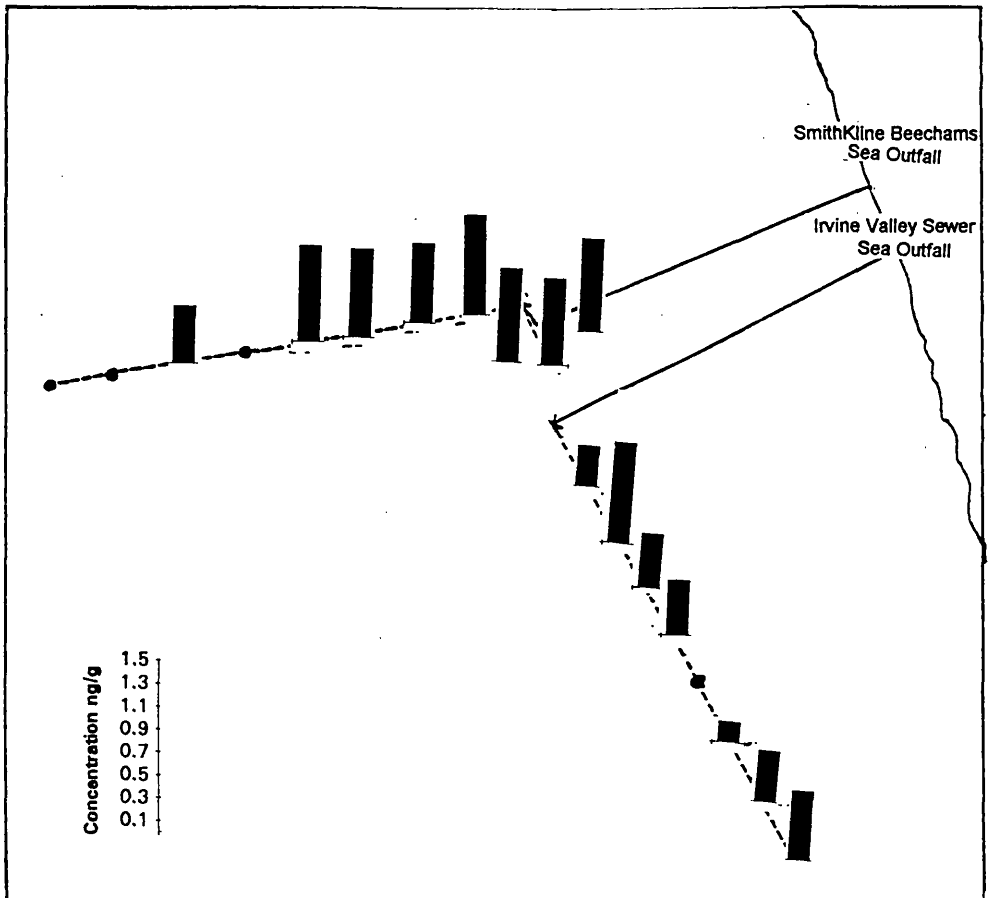


Figure 47 Irvine Bay Grab Survey: PP DDT Normalised to 1% TOC (ng/g Dry Weight)

GVS Sea Outfall
ICI Sea Outfall
IVS Sea Outfall
Diving Survey
Concentration ng/g
3.5
3
2.5
2
1.5
1
0.5
0
2 km

Figure 48 Irvine Bay Diving Survey: PP DDE Normalised to 1% TOC (ng/g Dry Weight).

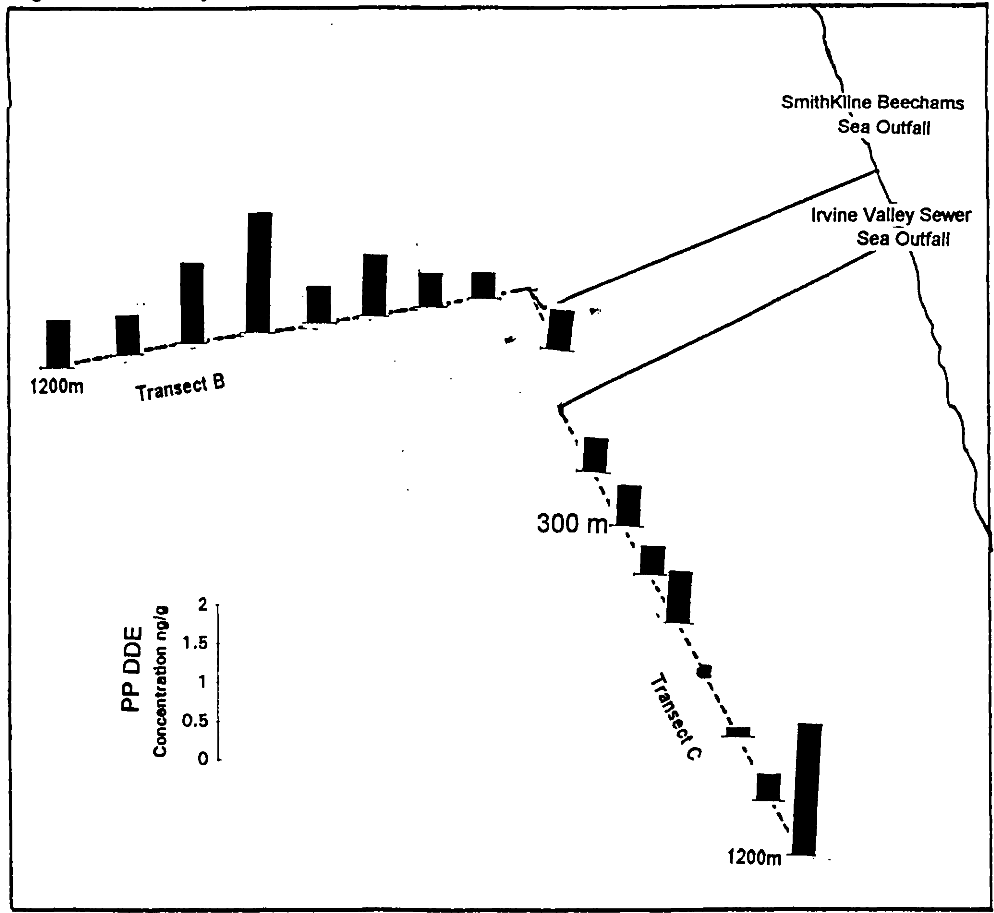


Figure 49 Irvine Bay Grab Survey: PP DDE Normalised to 1% TOC (ng/g Dry Weight)

Figure 50 Irvine Bay Diving Survey: γ HCH Normalised to 1% TOC (ng/g Dry Weight).

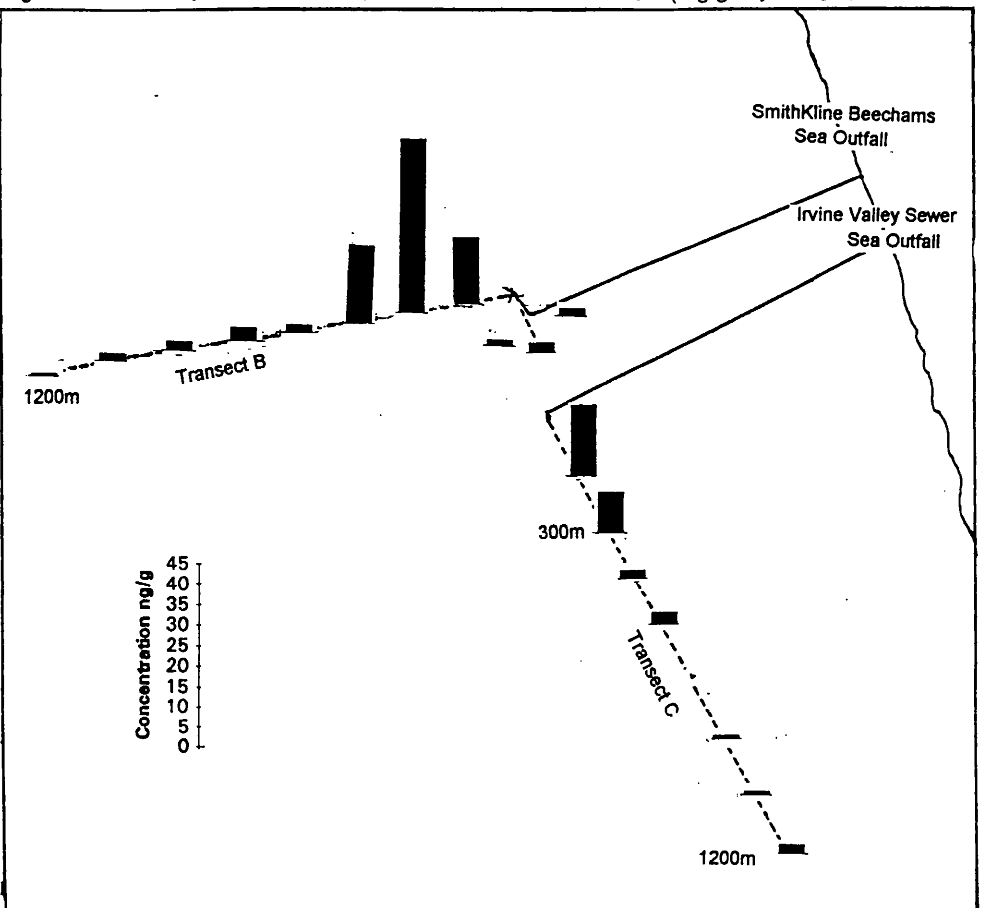


Figure 51 Irvine Bay Day Grab Survey: γ HCH Normalised to 1% TOC (ng/g Dry Weight).

Figure 52 Irvine Bay Diving Survey: Total PCBs Normalised to 1% TOC (ng/g Dry Weight).

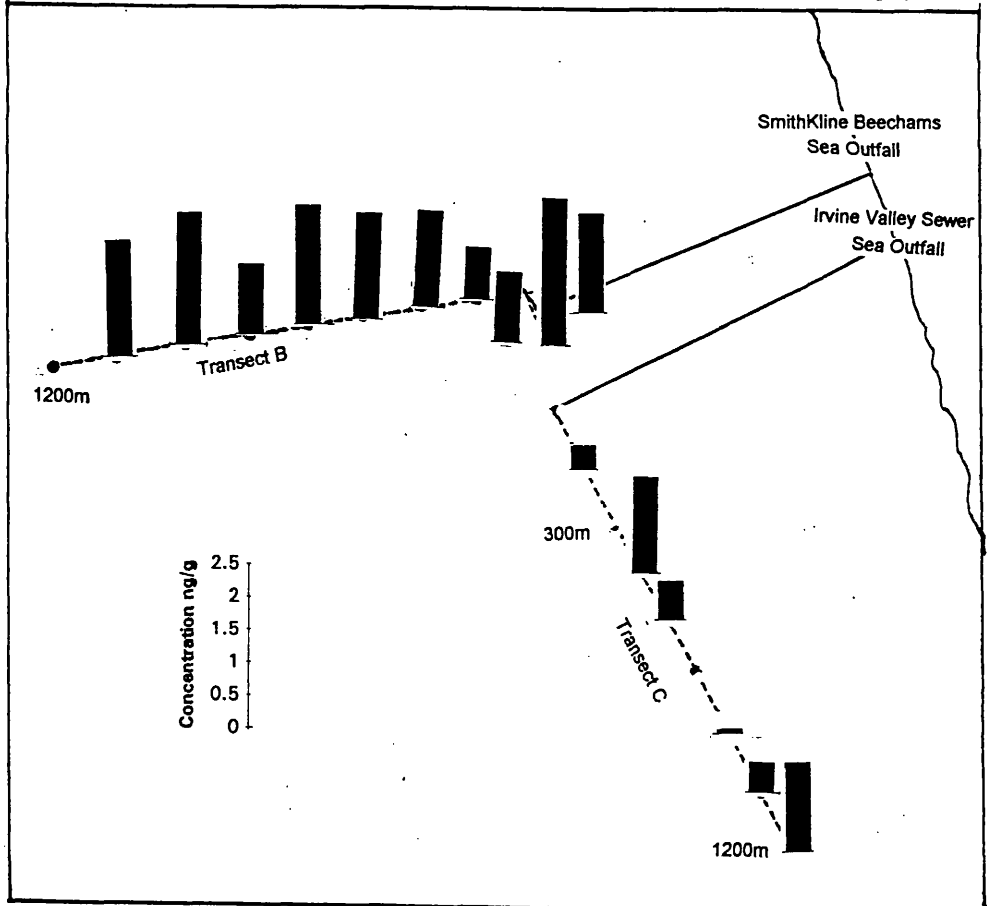


Figure 53 Irvine Bay Day Grab Survey: Total PCBs Normalised to 1% TOC (ng/g Dry Weight).

The spatial distribution of each of the organochlorine compounds is similar. The spatial distribution confirms the valley sewers as significant sources of these contaminants. The highest concentrations are detected at sites close to the valley sewers or with a high percentage of fine particles.

The concentrations of total Permethrin recorded close to Irvine Valley Sewer (sites B21, B2, B24 and C9) are comparable with those recorded in the Annick Water immediately down stream of Stewarton STW. The levels recorded in the sediment rapidly decrease with increasing distance from the outfall. 1 % organic carbon normalised total permethrin concentrations are below 5 ng/g at all sites greater than 1000m from the either of the valley sewers.

In the effluent from both valley sewers the cis and trans permethrin were present at a mean ratio of 38:62. This is very similar to the ratio of the technical product (40 :60), this ratio is slightly lower than the mean ratio of 44:56 which was observed in the effluent from Stewarton STW. The effluent from the valley sewers receives only coarse grit removal and not biological treatment as carried out at Stewarton STW. This difference in treatment may account for the slight variation in isomeric ratios. The mean isomeric ratio observed in the sediment was 49:51. This is higher than observed in the effluent and reflects the greater environmental stability of the cis isomer. Unlike sediment from the Annick Water no increase in the cis : trans ratio is observed with increasing distance from the outfalls. Possible explanations for this may be a much slower rate of degradation in the marine environment or more probably that sediment transport is the major route of permethrin distribution in the Annick Water and sediment from the lower reaches of the Annick Water contain permethrin which has been in the environment for a longer time resulting in a higher cis:trans ratio.

The organic carbon normalised concentrations of Dieldrin, DDT, and metabolites of DDT are slightly lower than those recorded in sediments from the Annick Water while those of PCB's are of a similar level. The concentrations of lindane recorded at sites close the outfalls were an order of magnitude higher than those recorded in the Annick Water.

A considerable number of studies have been conducted on the concentrations of PCBs in marine sediments around the UK and Europe while few studies have been conducted on the occurrence of organochlorine insecticides in marine sediments. The author is not aware of any studies on the occurrence of pyrethroid insecticides in marine sediments. Table 125 summarises literature data on the concentrations of organochlorine compounds detected in marine sediments from around the UK and Europe.

Table 125 Summary of the Concentrations of Organochlorine compounds detected in European Marine Sediments.

Location	Year	%TOC	Permethrin	Dieldrin	DDT	HCH	PCB	Reference
Inner Firth of Clyde	989	3.3 - 4	ND	1.3 - 3.6	1.2 - 13 DDT 5.9 - 15 DDD 2.2 - 5.1DDE	0.3 - 0.5 γ	16 - 45	Kelly & Campbell1995
Firth of Clyde - Incmarnock Water	1989	1.4 - 4.2	ND	0.8 - 3.4	0.4 - 4 DDT 2.6 - 8.0 DDD 1.5 - 2.2 DDE	0.1 - 0.2 γ	5.1 - 14	
Mid/South Firth of Clyde	1989	0.9 - 3.1	ND	0.5 - 1.9	0.1 - 1.7 DDT 1 - 5.4 DDD 0.7 - 2.1 DDE	0.1 - 0.4 γ	1.8 - 6.9	
Outer Firth of Clyde	1989	0.5 - 1.4	ND	0.1 - 0.6	0.1 - 0.2 DDT 0.3 - 1.9 DDD 0.1 0.9 DDE	0.1 -0.2 γ	0.5 - 6.7	
Clyde Estuary	1993	ND	ND	ND	ND	ND	68 - 119	D.Pirie unpublished results
Cart Estuary	1993	ND	ND	ND	ND	ND	43 - 567	
Liverpool Bay	1988	ND	ND	ND	ND	ND	13.7 - 37.9	Camacho-Ibar *et al* 1996
Inner Thames Estuary	1993	ND	ND	0.4 - 2.1	0.1 - 5.4 DDT 0.3 - 1.5 DDD 0.1 - 0.9 DDE	ND	3 - 40	Scrimshaw *et al* 1995
Tyne Estuary	1990	ND	ND	ND	ND	ND	6.1 - 81	MAFF 1993
Humber	1990	ND	ND	ND	ND	ND	3 - 7.6	
Cardigan Bay	1990	ND	ND	ND	ND	ND	0.2 - 3.9	
Morecambe Bay	1990	ND	ND	ND	ND	ND	0.2 - 4.7	
Humber, Tees, Tweed and Ribble Estuaries	1990	ND	ND	ND	ND	ND	0.2 - 20	ENDS 1993
Tyne, Wear and Mersey Estuaries	1990	ND	ND	ND	ND	ND	20 -100	
North Sea	1986	0.1 - 15	ND	ND	0.01 - 0.13DDE	0.01 - 0.23 α 0.01 - 0.16 γ	0.1 - 4.4	Knickmeyer & Steinhart 1988
	1987							*al* 1990

ND - Not Determined.

The concentrations of contaminants detected in sediments from Irvine Bay are in agreement with those reported for marine sediments from coastal areas of the UK and Europe.

The concentrations of dieldrin are similar to those found by other workers (Kelly & Campbell, 1995) in sediments from a wider survey of the Firth of Clyde. At sites close to the outfalls concentrations between 1 and 3 ng/g were detected. These concentrations are an order of magnitude lower than those found on the Clyde sludge dumping ground at Garrochhead (Kelly & Campbell *et al*., 1995) and are comparable to those found in the Inner Thames Estuary (Scrimshaw & Lester., 1995).

5.5.3 Comparison with Quality Standards

As indicated previously in section 4.6.5 the UK government has opted to set receiving waters environmental quality standards (EQS) rather than set limit values for the concentrations of contaminants in effluents. The UK EQSs for saline waters for the compounds which have been frequently detected in the effluent from the Valley sewers is summarised in Table126.

Table 126 UK Environmental Quality Standards For Organochlorine Compounds in Saline Waters.

Compound	Environmental Quality Standard
Total permethrin	10 ng/l 95 percentile
Dieldrin	10 ng/l
pp DDT	10 ng/l
Total DDT	25 ng/l
Total HCH	20 ng/l

To determine the probable concentrations of target analytes in the receiving waters of Irvine Bay for comparison with EQSs calculations are made using the measured effluent concentrations with theoretical dilution models.

The use of a mixing zone approach has become well established and accepted (Haig *et al*., 1989; Mackay *et al* 1986; and Water Authorities Association 1986) when considering discharges to the marine environment. The mixing zone is an area where the effluent may receive both initial dilution as it rises to the water surface and secondary dilution by diffusion and currents, the concentration of contaminants may exceed the environmental quality standards within the mixing zone. Extensive hydrographic work carried out by CRPB has estimated that the minimum dilution received by the effluents under consideration on entering the marine environment is 50 (Miller, CRPB pers comm.). Typical concentrations of contaminants in Irvine Bay at the edge of the mixing zone were estimated using this worst case dilution with median concentration of contaminants detected in the effluents from the valley sewers. Estimates of the maximum concentration at the edge of the mixing zone were calculated using the worst dilution and the maximum concentration recorded in effluent from the valley sewer. These estimates are listed in Table 127.

Table 127 Estimated Concentrations of Organochlorine Compounds at the Edge of the Mixing Zones of IVS and GVS.

	Irvine Valley Sewer		Garnock Valley Sewer	
Compound	Estimated typical Concentrations (ng/l) at edge of mixing zone	Estimated maximum concentrations (ng/l)at edge of mixing zone	Estimated typical Concentrations (ng/l) at edge of mixing zone	Estimated maximum concentrations (ng/l) at edge of mixing zone
cis permethrin	6.6	25.1	5.1	40
trans permethrin	9.9	40.3	10.6	55
total permethrin	16.5	65.4	15.7	95
dieldrin	0.4	1.6	0.1	0.3
pp DDE	0.1	0.3	0.1	0.1
pp DDD	0.1	0.1	0.1	0.1
pp DDT	0.2	1.1	0.2	1.5
Total DDT	0.4	1.5	0.4	1.7
α HCH	0.1	0.1	0.1	1.0
β HCH	0.2	0.5	0.1	0.8
γ HCH	0.6	2.1	0.6	2
total HCH	0.9	2.7	0.8	3.8

In typical conditions the estimated concentrations of permethrin at the edge of the mixing zone at both valley sewers are slightly in excess of the EQS. In adverse conditions the estimated concentrations of permethrin at the edge of the mixing zone are approximately 5 and 10 times higher than those permitted by the EQS.

The estimated concentrations of dieldrin, pp DDT, total DDT, individual HCH isomers and total HCH at the edge of the mixing zones of both Irvine and Garnock valley sewer in typical and adverse conditions were approximately an order of magnitude lower than those permitted by the EQS.

As indicated in section 4.6.5 at present the UK does not at present set sediment quality standards. However, a number of marine monitoring groups have recognised the need for a method of classifying sediment contaminant concentrations and a number of groups/organisations have suggested guideline concentrations for contaminants in marine sediments.

Following the Piper - Alpha incident in the North Sea SOAEFD defined a series of concentration guidelines in order to categorise the levels of chlorinated biphenyls in marine sediments (Wells *et al.*, 1989). These guidelines have been used to categorise sediments from around the UK coast (MAFF,1993) and have become widely accepted as an appropriate method of classifying PCB concentrations in marine sediments.

The Florida Department of Environmental Protection (F.D.E.) have recently developed effects based sediment quality assessment guidelines (SQAG) for 34 priority substances in Florida coastal waters (Florida Department of Environmental Protection, 1994). These guidelines have been developed using the weight of evidence approach (WEA). Using this approach a database is compiled containing information generated by the three approaches to the establishment of effects-based sediment quality guidelines: the equilibrium partitioning approach, the spiked-sediment toxicity approach, and the evaluation of matching sediment chemistry and biological effects data. The data in the database is weighted and used to compile the sediment quality assessment guidelines. These quality guidelines are designed for the evaluation of marine sediments around Florida. The Florida Department of Environmental protection SQAG for compounds which were frequently detected in sediments from Irvine Bay are listed in Table 128. Extreme caution must be exercised when comparing these guidelines to marine sediments from cold northern waters such as Irvine Bay.

Using similar methods the Joint Monitoring Group of the Oslo and Paris Commissions have also derived from studies which contained biological effects studies (Fleming et al., 1995), standard sediment concentrations below which biological effects are unlikely to occur. The majority of studies were North American/Canadian and as previously indicated the values should be used with caution.

These guideline sediment assessment concentrations are summarised below in Table 128. The SOAEFD/MAFF guideline do not account for sediment bound organic carbon, the FDEP and JMP guidelines are both for 1%TOC normalised sediments.

Table 128 Summary of Marine Sediment Quality Assessment Guidelines.

	SOAEFD / MAFF	F.D.E. P		JMP
Compound	Sediment Categories ng/g dry weight	Threshold effects level (TEL) ng/g dry weight	Probable effects level (PEL) ng/g dry weight	Sediment concentrations below which biological effects unlikely to occur. ng/g dry weight
Permethrin	NA	NA	NA	NA
Lindane	NA	0.32	0.99	5 - 50
Dieldrin	NA	0.72	4.3	5 -50
pp DDE	NA	2.1	374	NA
pp DDD	NA	1.2	7.8	NA
pp DDT	NA	1.2	4.8	NA
Total DDT	NA	3.9	51.7	NA
PCBs	<0.2 contamination not detectable 0.2 - 20 slightly contaminated 21 -100contaminated >100 heavily contaminated	21.6	189	1-10

The concentrations of dieldrin determined at sites greater than 1000M from the outfalls are all lower than the FDEP threshold effects concentrations. The concentrations of dieldrin at all sites are significantly lower than the FDEP predicted concentration at which biological effects occur, and below the JMP lowest concentration range at which biological effects occur. Concentrations slightly higher than the threshold effects concentration are observed at sites close to the outfalls, but this enhancement of dieldrin concentrations is not likely to result in widespread adverse biological effects.

The concentrations of DDT and metabolites of DDT determined at most sites were lower the FDEP threshold effects level and at all sites the concentrations are lower than the FDEP predicted biological effects concentration. As per dieldrin slight enhancement is observed at sites close to the outfalls but comparison with guideline values suggests that DDT and its metabolites are not significant contaminants in Irvine Bay and are unlikely to result in adverse biological effects.

All of the sites within Irvine Bay are classified as slightly contaminated by PCBs using the SOAEFD/MAFF classification scheme. All of the sites are in the lower region of this category. The organic carbon normalised concentrations are similar throughout the Bay and consistent with those reported by other studies (Kelly & Campbell, 1995). No significant enhancement of PCB concentrations is observed at sites close to the outfalls. These results confirm the conclusion of Kelly & Campbell that Irvine Bay is not a significant source of PCBs within the Firth of Clyde and adds weight to their suggestion that the sludge dumping at Garroch Head is the major source of PCBs within the Firth of Clyde. The PCB concentrations recorded in Irvine Bay sediments are an order of magnitude lower than the FDEP threshold effects concentration and in the lower portion of the JMP concentration range at which adverse biological effects are unlikely.

Lindane concentrations at most Irvine Bay sites are greater than the FDEP threshold and probable effects concentrations. Sites greater than 1000m from the outfalls of valley sewers are below the JMP concentration range below which biological effects are unlikely to occur. Concentrations in sediments close to Irvine Valley sewer are significantly enhanced and in the upper range of the JMP concentration band where biological effects are unlikely to occur. The concentrations of lindane determined in Irvine Bay are an order of magnitude higher than concentrations reported by Kelly& Campbell in sediments from the Firth of Clyde, and higher than those recorded by Kelly & Campbell on the sludge dump site at Garrochhead. Concentrations recorded close to Irvine Valley sewer are an order of magnitude higher than those recorded by Kelly & Campbell on the sludge dumpsite at Garrochhead. This confirms Kelly & Campbell's finding that there is a source of lindane in Irvine Bay and suggests that inputs of lindane from IVS are significant not only to Irvine Bay but to the wider Firth of Clyde.

No sediment standards/guideline values could be found for Permethrin. However, the sediment quality standards calculated for permethrin using the equilibrium partitioning approach in section

4.6.5 ranged from 0.13 - 245 ng /g with a median value of 55 ng/g. Additionally sediment toxicity studies on freshwater invertebrates discussed in section 4.6.6 found sediment bound permethrin to be acutely toxic at 40ng/g. Carbon normalised permethrin concentrations of 20-94 ng/g were observed to have a severe biological impact of the benthic fauna of the Annick Water between Stewarton STW and Chapeltoun and carbon concentrations of 2-20 ng/g were observed to have a moderate impact on the benthic fauna of the Annick Water. The synergistic and negative insecticidal temperature coefficient properties of permethrin disscussed for the Annick Water catchment are also relevant for the marine environment. Comparing the observed biological effects in the Annick Water with the concentrations of sediment bound permethrin in Irvine Bay suggests they will be acutely toxic to marine fauna at two sites (B21 and C9) within 1000m of the IVS, and have a significant adverse effect on marine fauna at all sites within a 1000m of both outfalls.

5.5.4 Comparison with Biological Effects

Marine benthic fauna are widely used as indicators of environmental quality (North sea Quality Status Report 1993). As benthic fauna live permanently in or on a substrate and integrate the effects of the various environmental conditions they are widely used in marine monitoring programs to assess the quality of the marine environment. As indicated earlier the Clyde River Purification Board has established a network of sites in Irvine Bay which it regularly monitors for benthic fauna. The most recent macrofaunal results available are from the analysis of grab samples collected in 1992, and core samples collected by divers in 1993. The results of macrofaunal analysis carried out by CRPB biologists on these samples are presented in Table 129.

Table 129 Results of Macrofaunal analysis of Sediments from Irvine Bay.

1992 Sediments collected using Day Grab		1993 sediments collected by divers	
Site No.	Shannon-Weiner ($\log_2$) Indices	Site No.	Shannon-Weiner ($\log_2$) Indices
J	3.91	B2	3.62
I	4.63	B21	1.57
H	4.81	B24	2.64
F	4.12	B25	2.72
J1	4.14	B29	1.26
L8	1.86	B34	2.30
L7	4.55	B35	3.05
L81	3.94	B36	2.91
L6	3.6	C9	0.73
Q2	3.46	C14	1.42
Q1	4.23	C26	1.50
Q	2.8	C27	NA
P	4.77	C31	NA
Z	4.19	C32	NA
E	4.35	C33	NA
R1	3.97	I1	NA
C	4.55	I6	NA
AB1	3.47	I7	NA
AB3	5.51		

Benthic fauna results may be presented using a variety of index e.g. Faunal abundance, diversity, Brillouin Index, Shannon-Weiner Index, Simpson's Index and William's Index. The number and diversity of benthic infauna found in coastal bays within the UK is limited by the food available. Organic enrichment of a bay causes both a change in the diversity and abundance of the benthic fauna found in the environment. For this reason simple indices such as the faunal abundance and species richness which measure only one of these parameters are of limited use when assessing the impact of a discharge such as Irvine Valley Sewer.

The Shannon-Weiner Index was selected as the biological indice most appropriate to monitor the effects of Irvine and Garnock Valley Sewers (O' Rielly, pers comm.). This index estimates the diversity per individual of a sample by examining the proportional abundances of all the different species. This takes into account both the number of species present and their differing abundance.

The typical range of values for this index are between 0 and 6. Diversity values greater than 3 are regarded as normal (unimpacted) for sediment samples from UK coastal bays such as Irvine Bay. Diversity values between 2 and 3 are regarded as slightly reduced diversity (Mild impact), values between 1 and 2 are regarded as moderately impacted . Diversity values lower than 1 are regarded as severe impact (greatly reduced diversity) (O' Rielly, pers comm.).

From this it can be seen that the areas of biological impact follow a similar pattern to the pattern of organochlorine contamination and organic carbon. The areas of highest impact were close to the discharge points of both valley sewers. Greater biological effect was observed at Irvine Valley sewer. Only one site (C9) within 500 M of the discharge was severely impacted, all the sites within 1000M of the discharges were moderately impacted. Sites between 1000 and 1500M exhibited signs of mild impact, sites greater than 1500M from the outfalls show no signs of impact. At present it is not possible to draw clear cause and effect conclusions between specific contaminants and these effects.

These observations of possible biological effects on the fauna of Irvine Bay are in good agreement with the predicted effects from the application of the various sediment quality standards.

5.5.5 Conclusions

Organochlorine contaminants were detected in effluent from Irvine and Garnock Valley sewers and in sediment from Irvine Bay.

Contaminants found in the Irvine Bay sea outfall effluents include permethrin, HCH isomers, dieldrin, DDT and metabolites of DDT. This was similar to the composition of the Stewarton effluent. Permethrin and isomers of HCH were the compounds most frequently detected and at the highest concentrations.

The concentrations of dieldrin, DDT, metabolites of DDT and isomers of HCH detected in the effluent from the valley sewers were unlikely to cause a breach of the relevant environmental quality standards for saline waters outside the mixing zone. The concentration of permethrin detected in the effluent from the valley sewers in typical conditions were likely to cause a slight breach of the EQS at the edge of the mixing zone. In adverse conditions the concentrations of permethrin in the effluent from both valley sewers were likely to result in permethrin concentrations at the edge of the mixing zone approximately 5 and 10 times higher than those permitted by the EQS.

Permethrin, dieldrin, HCH isomers, DDT, DDT metabolites and PCBs were detected in sediments from Irvine Bay. Permethrin and γ HCH were the major contaminants detected. The spatial distribution of organic carbon normalised contaminants indicated that the valley sewers are the major sources of these contaminants within Irvine Bay. Slightly elevated concentrations of dieldrin, DDT and metabolites of DDT were also observed at sites close to the valley sewers indicating that the valley sewers are minor sources of these compounds.

PCBs were detected at low concentrations at all sites from within Irvine Bay. No significant elevation in PCB concentrations was observed at sites close to the valley sewers, indicating that the valley sewers are not significant sources of PCBs.

The concentrations of dieldrin, isomers of HCH, DDT, metabolites of DDT and PCBs were consistent with those reported by other authors both for sediments from the Firth of Clyde and other European estuaries. Sediments from Irvine Bay are classified as slightly contaminated for PCBs.

The concentrations of PCBs, dieldrin, DDT and metabolites of DDT detected in the sediments were below both FDEP and JMP sediment guideline/assessment concentrations at which adverse biological effects are expected. The concentrations of permethrin detected in sediments from within the mixing zone were similar to those shown in laboratory toxicity tests to have a toxic effect on freshwater organisms, the concentrations of permethrin detected in sediments outwith the mixing zone were an order of magnitude lower than those shown to have a toxic effect in laboratory based toxicity tests but similar to those demonstrated to have an adverse effect on the benthic fauna of the Annick Water.

Shannon -weiner diversity index values indicated that biological impact followed a similar pattern to the organochlorine contaminant levels, with the sites at which greatest impact was observed being the sites where the highest concentrations were detected. It was not possible to conclude if the biological impact was related to a specific contaminant.

Chapter 6

Conclusions

The objectives of the work reported in this thesis were:

1. to develop analytical methods for the determination of synthetic pyrethroids together with a range of persistent organochlorine contaminants in a variety of environmental matrices,

2. to estimate the precision and bias of the developed analytical methods,

3. to investigate the distribution and environmental significance of the discharge of synthetic pyrethroids and organochlorine contaminants from wool processing and textile industries in the Annick Water (Freshwater) and Irvine Bay (Marine) ecosystems.

Analytical methods were developed for the determination of the following compounds: cis permethrin, trans permethrin, α HCH, β HCH, γ HCH, pp DDT, pp DDD, pp DDE, op DDT, op DDD, op DDE, dieldrin, endrin, aldrin, alpha endosulphan, beta endosulphan, hexachlorobenzene, hexachlorobutadiene, and polychlorinated biphenyls. These methods were developed as tools for the determination of these compounds in river waters, sewage effluents, sediments, and biota.

The use of gas chromatography with electron capture and mass spectrometric detection for the final separation and detection of the target analytes was optimised and compared. The optimised gas chromatography separation conditions are summarised below :

Oven Temperature Program -	70 ^{0}C hold for 1min
	70 - 180^{0}C at 20 ^{0}C/min hold at 180^{0}C for 2 min
	180-230^{0}C at 1.2 ^{0}C/min hold at 230^{0}C for 10min
	230-280^{0}C at 3.5 ^{0}C/min hold at 280 for 15 min
Injection Technique -	On-column
Column -	HP Ultra-2, length 50m, ID 0.2mm, film thickness 0.33μm
Carrier Gas -	Hydrogen

These conditions allowed the complete separation of all the target analytes in a single chromatographic analysis. However, the separation of polychlorinated biphenyls from the majority of the other target analytes was still recommended for matrices such as sediments and biota.

Electron capture detection and mass spectrometry with electron impact ionisation in scanning and selected ion monitoring modes were found to be useful for the detection of the target analytes in environmental matrices. Electron capture detection was found to be a sensitive, precise, robust and easy to use method of detection for the target analytes. Electron capture detection was selected as the most appropriate method of detection for the routine determination of the target analytes.

Mass spectrometry in electron impact ionisation scanning mode was found to be only useful as a means of unequivocal identification of high concentrations of the target analytes. Mass spectrometry in electron impact ionisation mass selected ion recording mode was found to be sensitive but not suitable for routine use. This technique was selected for the routine confirmation of analytical results. Mass spectrometry with negative chemical ionisation was demonstrated to have considerable promise as a detection method but at present was not robust or stable enough for routine use.

The preparation of analytical standards and methods of calibration were thoroughly investigated. It was found that the preparation of calibration standards can be a significant source of error in organic analysis. Tests showed that organic liquids are most accurately dispensed by using a microsyringe to dispense an approximate estimate of the required volume and then weighing the exact amount delivered. It is also recommended that all standards should be prepared by weight. The use of volumetric glassware such as standard flasks is not recommended for the preparation of standards. Internal standards such as trans heptachlor epoxide and decachlorobiphenyl were found to improve the precision of the analytical procedures. The electron capture detector was found to be non-linear, multi point internal standard calibrations specific for each analyte were found to be the optimum method of calibration.

TurbVap concentration was found to provide a fast efficient means of sample concentration and solvent removal. All of the target analytes were quantitatively recovered when using this method of concentration.

A range of adsorption chromatography techniques were optimised and evaluated for use as clean-up procedures for the target analytes. Adsorption chromatography with "active" silica gel was found to be suitable for the separation of PCBs from the majority of the target analytes. Aminopropyl bonded silica gel cartridges were found to be of limited use in the removal of coextracted material from sediment and biota extracts. Florisil cartridges were found to quick and easy to use and ideally suited to the removal of small amounts of coextracted material from extracts of river water samples. Acidic and basic treated alumina columns were found to time consuming to prepare and had a very low capacity for the removal of coextracted compounds. Acidic/Basic treated alumina was not considered suitable for the routine clean-up of environmental extracts. Neutral and silver nitrate treated alumina were both found to have excellent capacities for the removal of coextracted material from environmental extracts. Neutral alumina was particularly suited to the removal of lipid

material encountered in extracts from biota. Silver nitrate impregnated alumina was particularly successful in the cleanup of highly coloured extracts encountered in sediment analysis.

Liquid-liquid and solid phase extraction were investigated for the extraction of the target analytes from aqueous samples. For 1L aqueous samples double extraction with 50mls of hexane was determined as the optimum liquid-liquid extraction technique. Solid phase extraction was shown to be an excellent technique for the extraction of clean water samples (e.g. deionised water, tap waters). Practical difficulties were encountered when using vacuum manifold and crude positive pressure systems to apply this technique to the extraction of the target analytes from river waters. Solid phase extraction of environmental samples with significant amounts of suspended particles was only feasible using dedicated instruments such as the Zymark Autotrace. Using the Zymark Autotrace C8 bonded silica cartridges were found to provide optimum retention of the target analytes, optimum elution was determined as 2* 3ml portions of 50% acetone/hexane followed by elution with 2* 3ml portions of hexane.

Extraction solvent and extraction time were optimised for the soxhlet extraction of the target analytes from sediment and biota. Methyl tert butyl ether and 7 hours were determined as the optimum conditions.

These optimised analytical techniques were combined to form 5 analytical procedures for the determination of the target analytes in a range of environmental matrices. Routine quality control procedures to assure the performance of these procedures were put in place. The performance of these optimised procedures was shown to be excellent by thorough precision testing and participation in a variety of interlaboratory exercises.

The distribution and environmental significance of organochlorine and synthetic pyrethroid compounds following their discharge from the textile and wool processing industries in the Annick water and Irvine Bay ecosystems was fully investigated.

Organochlorine compounds were detected in the effluent from Stewarton STW, the Annick Water, sediments from the Annick Water and eels from the Annick water. Permethrin, isomers of HCH, dieldrin and metabolites of DDT were the compounds most frequently detected.

The concentrations of permethrin detected in the effluent from Stewarton STW were likely to cause a breach of the freshwater environmental quality standard for permethrin in the Annick Water.

Permethrin was also the most significant contaminant detected in sediment from the Annick Water. The spatial distribution of contaminants in the sediment indicated two sources of contamination: spillages from the wool processing factory, and the final effluent from Stewarton STW. The pattern of contaminants indicated that the STW was the major source of contamination.

Comparison with derived sediment environmental quality standards, published sediment toxicity data, and published environmental concentrations of organochlorine contaminants indicated that the concentrations of permethrin detected in the sediments of the Annick Water between the STW and Chapeltoun were acutely toxic to aquatic invertebrates. Invertebrate biotic index scores and fish population studies of the Annick Water confirmed that there was a significant adverse effect on the fauna of the Annick Water.

Permethrin was not detected in eels caught from either the Annick Water or the Glazert Burn, this may reflect the ability of the eels to metabolise permethrin. Significant concentrations of dieldrin were detected in eels from the Annick Water down stream of the STW. The concentrations of dieldrin detected in these eels suggest that regular consumption of eels may pose a threat to fish eating-birds, aquatic mammals and humans. PCBs were also detected in eels from the Annick Water downstream of the STW the concentrations were in the medium JMP category and close to those thought to have an on effect fish-eating birds and mammals. Concentrations of HCH isomers, DDT and DDT metabolites were also detected in eels from the Annick Water, these concentrations were below European consumption standards and similar to published concentrations in eels.

Organochlorine contaminants were also detected in the effluent from Irvine and Garnock Valley Sewers and in sediment from Irvine Bay. The contaminants detected were similar to those found in effluent from Stewarton STW.

The concentrations of dieldrin, DDT, metabolites of DDT and isomers of HCH detected in effluent from the valley sewers were unlikely to cause a breach of their environmental quality standards in

the receiving waters. The concentrations of permethrin detected in effluent from both valley sewers may cause a slight breach in the environmental quality standard.

Permethrin and γ HCH were the major contaminants detected in sediments from Irvine Bay. The spatial distribution of organic carbon normalised contaminants indicated that the valley sewers were the major sources of these contaminants within Irvine Bay. The concentrations of γ HCH and permethrin in sediments from within the mixing zones of the two valley sewers were similar to those shown likely to have an adverse effect on aquatic fauna.

Polychlorinated biphenyls were detected at low concentrations in all the sediments from within Irvine Bay. No significant elevation in PCB concentrations was observed at sites close to the valley sewers, indicating that the valley sewers are not significant sources of PCBs. This supports the supposition that sludge dumping at Garroch Head is the major source of PCBs within the Clyde Sea.

Shannon-Weiner biological diversity values were closely linked to concentrations of organochlorine contaminants. The sites with the highest concentrations of organochlorines were the sites at which the greatest biological impact was observed.

This study achieved its original aims, analytical methods suitable for the determination of trace quantities of organochlorine and pyrethroid contaminants were successfully developed, validated and applied to two environmental case studies. These studies successfully identified a range of contaminants from the textile and wool processing industries in effluents, river waters, sediment and biota. The concentrations and spatial distribution of these contaminants were compared with environmental quality standards, published toxicity data, published environmental concentrations and observed biological effects. This allowed an assessments of the potential hazard posed to the environment by the release of these chemicals to be made. This assessment was partially responsible for the introduction of more stringent discharge controls to the Annick water and the subsequent improvement in its biological quality.

The success of these environmental studies emphasises the need for a holistic approach when designing environmental monitoring programmes. Monitoring programmes should include both chemical and biological aspects so that where possible measured biological responses can be related to specific chemical exposures. Chemical monitoring should reflect the biogeochemical behaviour and physico-chemical characteristics of the target analytes. For example, monitoring of receiving waters is of limited value when assessing the potential impacts of lipophilic substances such as dieldrin and permethrin. In addition where possible chemical monitoring should not simply record total concentrations but try to assess the bioavailable fraction. The success of all monitoring is built on the implementation of a strong quality assurance system. Quality assurance is necessary to ensure that a program is adequate for the intended purpose and to provide an ongoing basis by which the quality of data is maintained. Demonstrably reliable and accurate environmental monitoring is an essential ingredient in the decision making which safeguards public health and improves the quality of the environment.

The work outlined in this thesis has covered only a very small number of the persistent organochlorine contaminants which pose a threat to our environment. Further research should investigate the possibility of including the determination of a number of these compounds alongside the determination of the contaminants considered in this thesis. Contaminants worthy of consideration for inclusion in such a multi residue scheme include: the planar PCBs, toxaphene, polychlorinated terphenyls, polchlorinated naphthalenes, polychlorinated paraffins, polybrominated biphenyls, polybrominated diphenylethers, polychlorinated camphenes, and tetrachlorobenzyltoluenes. In addition to information on other chlorinated contaminants further research is required on the bioavailability of contaminants within Irvine Bay. No biota from Irvine Bay were analysed in this study, further work is required to determine if the elevated concentrations of permethrin and γ HCH recorded in sediments close to the valley sewers are bioavailable and accumulating significantly in the fauna of Irvine Bay.

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APPENDIX 1

Substances governed by the dangerous substances directive (76/464/EEC)

LIST I substances	List II substances
Mercury	Lead
Cadmium	Chromium
Hexachlorohexane (all isomers)	Zinc
DDT (all isomers)	Copper
Pentachlorophenol (PCP)	Nickel
Carbon tetrachloride	Arsenic
Aldrin	Boron
Dieldrin	Iron
Endrin	pH
Isodrin	Vanadium
Hexachlorobenzene (HCB)	Tributyltin
Hexachlorobutadiene (HCBD)	Triphenyltin
Chloroform	PCSDS
Tetrachloroethylene	Cyfluthrin
Trichlorobenzene	Sulcofuron
Trichloroethylene	Flucofuron
1,2- Dichloroethane	Permethrin

APPENDIX 2

Red List Substances and Target Reductions : The following list of 36 substances comprises Annex 1A of the Final Declaration of the 3rd North Sea Conference

Substance	Target Reduction
Mercury	70 %
Cadmium	70 %
Copper	50 %
Zinc	50 %
Lead	70 %
Arsenic	50 %
Chromium	50 %
Nickel	50 %
Drins	50 %
HCH	50 %
DDT	50 %
Pentachlorophenol	50 %
Hexachlorobenzene	50 %
Hexachlorobutadiene	50 %
Carbon Tetrachloride	50 %
Trifluralin	50 %
Endosulfan	50 %
Simazine	50 %
Atrazine	50 %
Tributyltin Compounds	50 %
Triphenyltin Compounds	50 %
Azinphos ethyl	50 %
Azinphos methyl	50 %
Fenitrothion	50 %
Fenthion	50 %
Malathion	50 %
Parathion	50 %
Parathion methyl	50 %
Dichlorvos	50 %
Trichloroethylene	50 %
Tetrachloroethylene	50 %
Trichlorobenzene	50 %
1,2-Dichloroethane	50 %
Trichloroethane	50 %
Dioxins	70 %

APPENDIX 3

In addition to the commitment regarding the 36 substances in Annex 1A of the 3rd North Sea Conference Declaration, further common actions were agreed with respect to the reduction of inputs of specific substances and groups of substances.

1. **Pesticides-** to aim for a substantial reduction in the quantities of pesticides reaching the North Sea and thus, by 31/12/92, to strictly control their use and application and reduce, where necessary, emissions to the environment. Annex 1B part(c) lists 18 substances, employed as pesticides, the use of which must be strictly prohibited or banned:
   Aldrin, Atrazine, Carbon Tetrachloride, Chlordane, Chlorpicrin, 1,2 Dibromoethane, 1,2 Dichloroethane, Dieldrin, Endrin, Fluroacetic acid, Heptachlor, Hexachlorobenzene, Hexachorohexane, Mercury compounds, Nitrofen, Pentachlorophenol, Polychlorinated terpenes, Quintozene.

2. **PCBs-** to prevent PCBs and hazardous PCB substitutes from entering the marine environment including the phasing out of and destruction of all identifiable PCBs as soon as possible.

3. **Nutrients-** in applying the precautionary principle, to co-ordinate initiatives to reduce nutrient inputs, in order to meet the aim of a reduction of around 50% for inputs between 1985 and 1995 into areas where they are likely to cause pollution.

APPENDIX 4

Cyclodiene Insecticides

The insecticidal properties of this group of pesticides were discovered after the second World War. All of these compounds are obtained by variation of a Diels-Alder reaction with hexachloropentadiene. This group includes the compounds dieldrin, aldrin, endrin, isodrin, α and β endosulphan. These are very successful broad spectrum non-systemic insecticides used for the protection of a range of root crops, as sheep-dips, as mothproofing agents, and as timber preservative.

DDT

DDT is the common name given to dichlorodiphenyltrichloroethane. This compound was first prepared by Zeidler in 1874 but it's powerful insecticidal properties were not discovered until 1939 when Muller investigated DDT in his search for an effective mothproofing chemical (Cremlyn 1990). Crude DDT consists of 80% pp DDT and 20 % op DDT, only the pp form has significant insecticidal activity. pp DDT exhibits biological activity against a wide range of insects and in the fifties became the most widely used insecticide throughout the world. The major environmental metabolites of DDT are dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethene (DDE) both of these metabolites can exist as pp and op forms.

Hexachlorobenzene (HCB)

HCB is formed as a by-product of many chemical manufacturing processes. A large percentage of the HCB found in the environment is as a result of the production of chlorinated solvents, other HCB by-product sources include certain pesticide manufacturing plants.
World-wide HCB is used as a fungicide, and it is also used as a chemical intermediate in dye manufacture, in the synthesis of other organic chemicals, and as a wood preservative. In the UK it is not intentionally produced, nor is it approved for use as a pesticide.

Hexachlorobutadiene (HCBD)

HCBD is chiefly produced as a by-product from the manufacture of chlorinated hydrocarbons. It is used world-wide in the manufacture of rubber compounds and, to a lesser extent, in the manufacture of lubricants, as a gyroscope fluid, a heat transfer fluid, and a hydraulic fluid. Outside the UK it is used also to kill soil pests, but at present HCBD is not licensed for use as a pesticide within the UK.

Hexachlorohexanes

Hexachlorohexane was first prepared in 1912 by Van de Linden, however, its insecticidal properties were not discovered until 1942 (Cremlyn 1990) . Theoretically there are eight steroisomers of hexachlorohexane, five of which are present in the technical product. Technical HCH consists of 60-70 % α HCH, 5-12 % β HCH, 10 -15 % γ HCH, 6-10 % δ HCH, and 3-4 % ε HCH (Butte et al 1991). Only (HCH exhibits significant insecticidal activity. Technical HCH was introduced as an insecticide shortly after the end of the second World War, in 1979 the usage of technical HCH was restricted (United Nations, New York 1994). At present the major usage of HCH is as Lindane. Lindane contains greater than 99% HCH. The α, $\beta$and γ isomers of HCH are found widely in the environment. Lindane is a broad spectrum insecticide, which is widely used in wood preservation, agriculture, horticulture, food storage, animal husbandry, public hygiene and in the home.

Synthetic Pyrethroids

Natural pyrethrins such as the insecticide Pyrethrum obtained from the flower heads of _Chrysanthemum cinerariaefolium_ were among the earliest insecticides used by man (Alloway & Ayres 1993). These pyrethroids displayed high toxicity towards insects with low mammalian toxicity and rapid metabolism to non toxic products. Unfortunately, these compounds were readily degraded by sunlight and this severely restricts their usefulness. In the late forties workers (Elliott and co-workers Rothamsted Experimental Station and Sumitomo in Japan) began investigating the synthesis of photostable synthetic derivatives of natural pyrethrin. These derivatives are commonly referred to as the synthetic pyrethroids. Permethrin, cypermethrin, deltamethrin and bifenthrin were among the first synthetic pyrethroids to be developed commercially (Hill, 1989). All pyrethroids are structurally similar and share a common mode of biological action, namely disruption of voltage-gated sodium channels on nerve axons. Pyrethroids typically exists as complex mixtures of optical and geometric isomers with different isomers exhibiting different biological activity (Hassall, 1990). Pyrethroids have a very broad spectrum of anthropoid activity at exceptionally low dosages, for example 5 g of deltamethrin can protect the same area of cereals from aphid damage as 1Kg of organophosphate insecticide, or 15 kg of deltamethrin can treat as many houses for mosquito control as one tonne of DDT (Hill, 1990). In the two decades since their introduction in the mid seventies pyrethroids have become the fastest growing chemical group of insecticides (Cremlyn, 1991). Their broad spectrum of activity and low dosage rates has resulted in their usage in a wide variety of applications such as grain and food stores, cereal crop protection, soft fruit and vegetable protection, animal health protection, mothproofing, water mains protection, public health protection and a variety of amenity and household outlets.

PCBs

Polychlorinated biphenyls (PCBs) are complex mixtures of chlorine substituted biphenyls. They are prepared by reacting biphenyl with chlorine in the presence of a catalyst such as iron (III) chloride. The resultant product is not a pure compound but a complex mixture of congeners with differing degrees of chlorination (Tanabe, 1988). These mixtures were marketed under trade names such as Aroclor, Phenoclor and Clophen. One of the largest manufacturers of PCBs was Monsanto (Law, 1993). Their nomenclature typically designates Aroclor 1242 as the fraction containing 42 % of chlorines, Aroclor 1254 as 54 % chlorine and Aroclor 1268 as 68 % chlorine. PCB formulations have been prepared industrially since 1929, the highest production was in the sixties and early seventies. Environmental concerns led to severe restrictions on their sale and manufacture in most industrialised countries in the mid to late seventies, however, production continued in some countries such as the former Soviet Union until the early nineties (Colburn, 1996). Within the UK PCB production commenced in 1954 and ceased in 1977, within that period a total of 66,748 tonnes were produced (Harrad et al., 1994).

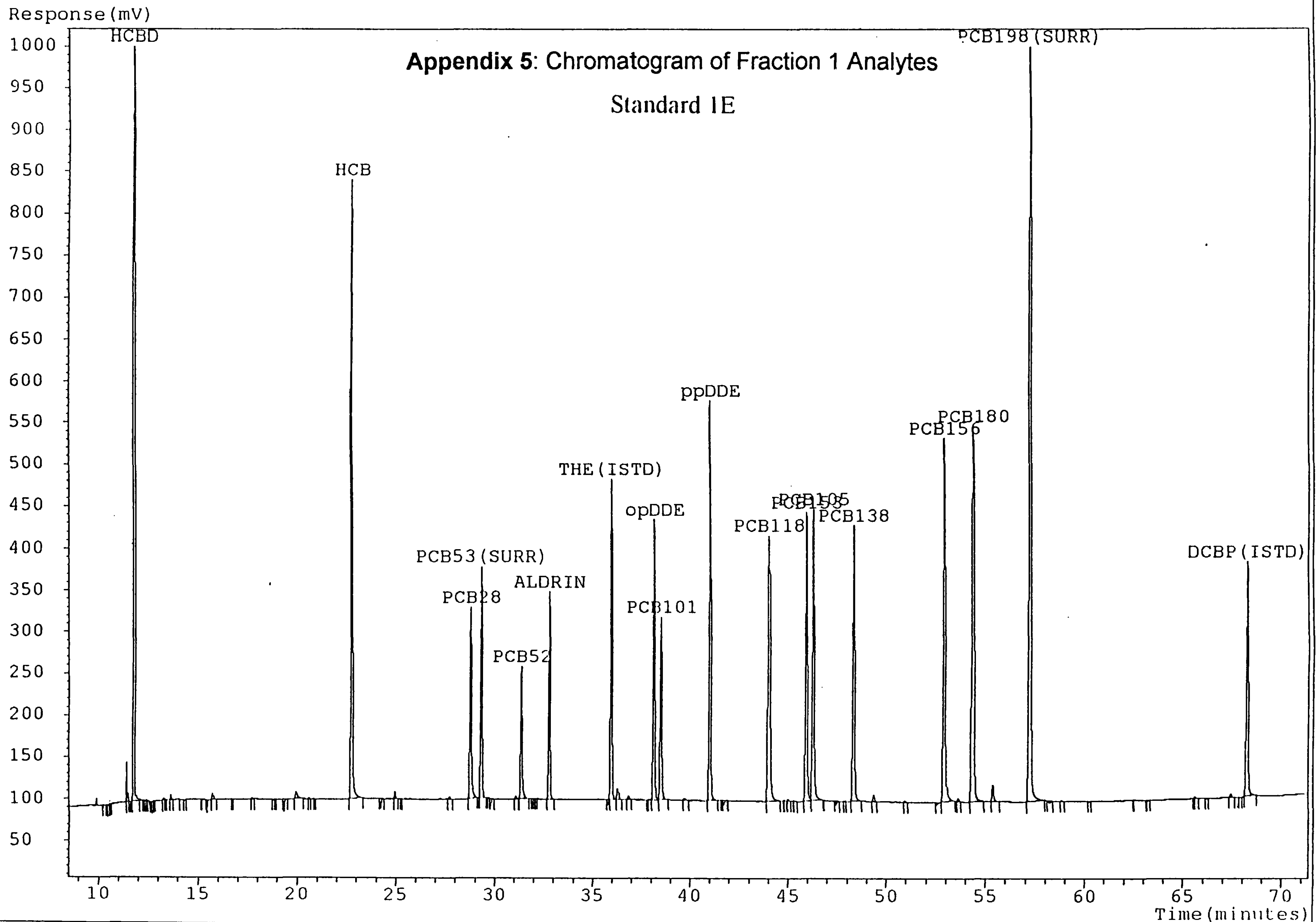
PCBs are exceptionally stable compounds that are non flammable (destruction by burning requires a temperature of over 1300 °C) and highly resistant to oxidation, acids, bases, and other strong chemical reagents, in addition they are excellent electrical insulators (Tatsukawa & Tanabe, 1987). Because of these characteristics, PCBs have been used in a variety of industrial applications. Prior to 1970 PCBs were used primarily in closed or semiclosed systems in electrical transformers, capacitors, heat transfer systems, and hydraulic fluids. They were used to a minor extent in paints ,adhesives, caulking compounds, plasticisers, inks, lubricants, carbonless copy paper, sealants, coatings, and dust control agents.

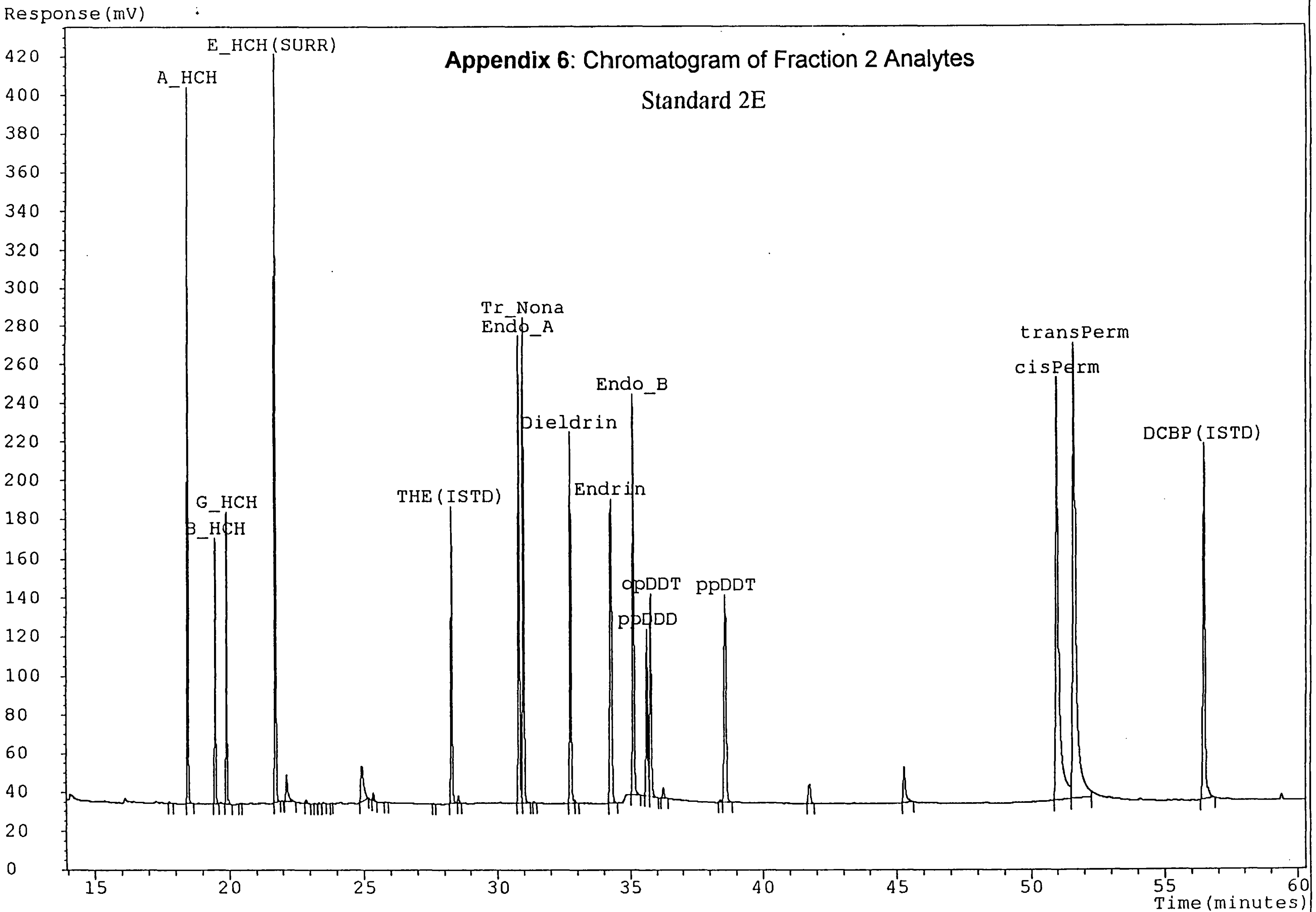
PCBs have been shown to be ubiquitous environmental contaminants with residues having been detected in all parts of the globe (Bommanna et al., 1994). As PCBs are complex mixtures of theoretically 209 congeners it is not analytically possible to accurately quantify all of these individual congeners (Duinker et al., 1988). To avoid confusion over which congeners should be quantified and allow comparison of result from different research groups the International Council For the Exploration of the Seas have recommended seven congeners with chlorine numbers from 3 -7 which should be monitored in environmental samples. These so called ICES 7 congeners are listed below:

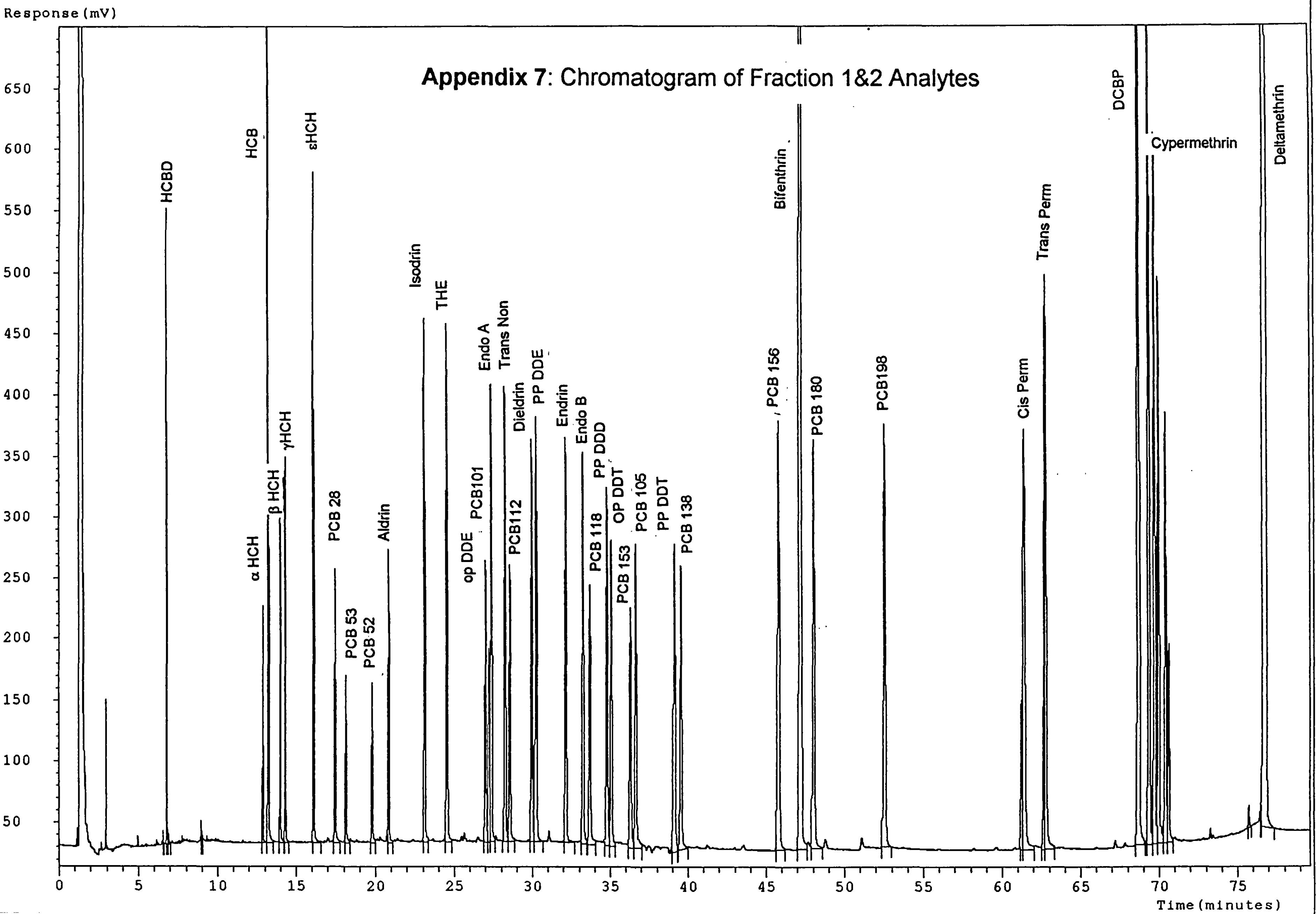
Table 130 ICES 7 congeners plus additional congeners recommended by Quasimeme.

Congener No.	Chemical Structure	Group
28	2,4,4' trichlorobiphenyl	
52	2,2',5,5' tetrachlorobiphenyl	ICES 7 congers
101	2,2',4,5,5' pentachlorobiphenyl	
118	2,3',4,4',5 pentachlorobiphenyl	
138	2,2',3,4,4',5' hexachlorobiphenyl	
153	2,2',4,4',5,5' hexachlorobiphenyl	
180	2,2',3,4,4',5,5' heptachlorobiphenyl	
105	2,3,3',4,4' pentachlorobiphenyl	Additional congeners
156	2,3,3',4,4',5 hexachloro biphenyl	recommended by Quasimeme

In addition to these congeners interlaboratory schemes recommended the determination of more toxic congeners such as CBs 156 and 105 these are also listed in Table 130.


Appendix 5: Chromatogram of Fraction 1 Analytes
Standard 1E
Response (mV)
1000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
HCBD
HCB
PCB53 (SURR)
PCB28
PCB52
ALDRIN
THE (ISTD)
opDDE
PCB101
ppDDE
PCB118
PCB105
PCB153
PCB138
PCB156
PCB180
PCB198 (SURR)
DCBP (ISTD)
10
15
20
25
30
35
40
45
50
55
60
65
70
Time (minutes)


Appendix 6: Chromatogram of Fraction 2 Analytes
Standard 2E
Response (mV)
420
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
A_HCH
E_HCH (SURR)
G_HCH
B_HCH
THE (ISTD)
Tr_Nona
Endo_A
Dieldrin
Endo_B
Endrin
opDDT
ppDDD
ppDDT
transPerm
cisPerm
DCBP (ISTD)
15
20
25
30
35
40
45
50
55
60
Time (minutes)


Appendix 7: Chromatogram of Fraction 1&2 Analytes
Response (mV)
Time (minutes)
650
600
550
500
450
400
350
300
250
200
150
100
50
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
HCBD
HCB
α HCH
β HCH
γHCH
εHCH
PCB 28
PCB 53
PCB 52
Aldrin
Isodrin
THE
op DDE
PCB101
Endo A
Trans Non
PCB112
Dieldrin
PP DDE
Endrin
Endo B
PCB 118
PP DDD
PCB 153
OP DDT
PCB 105
PP DDT
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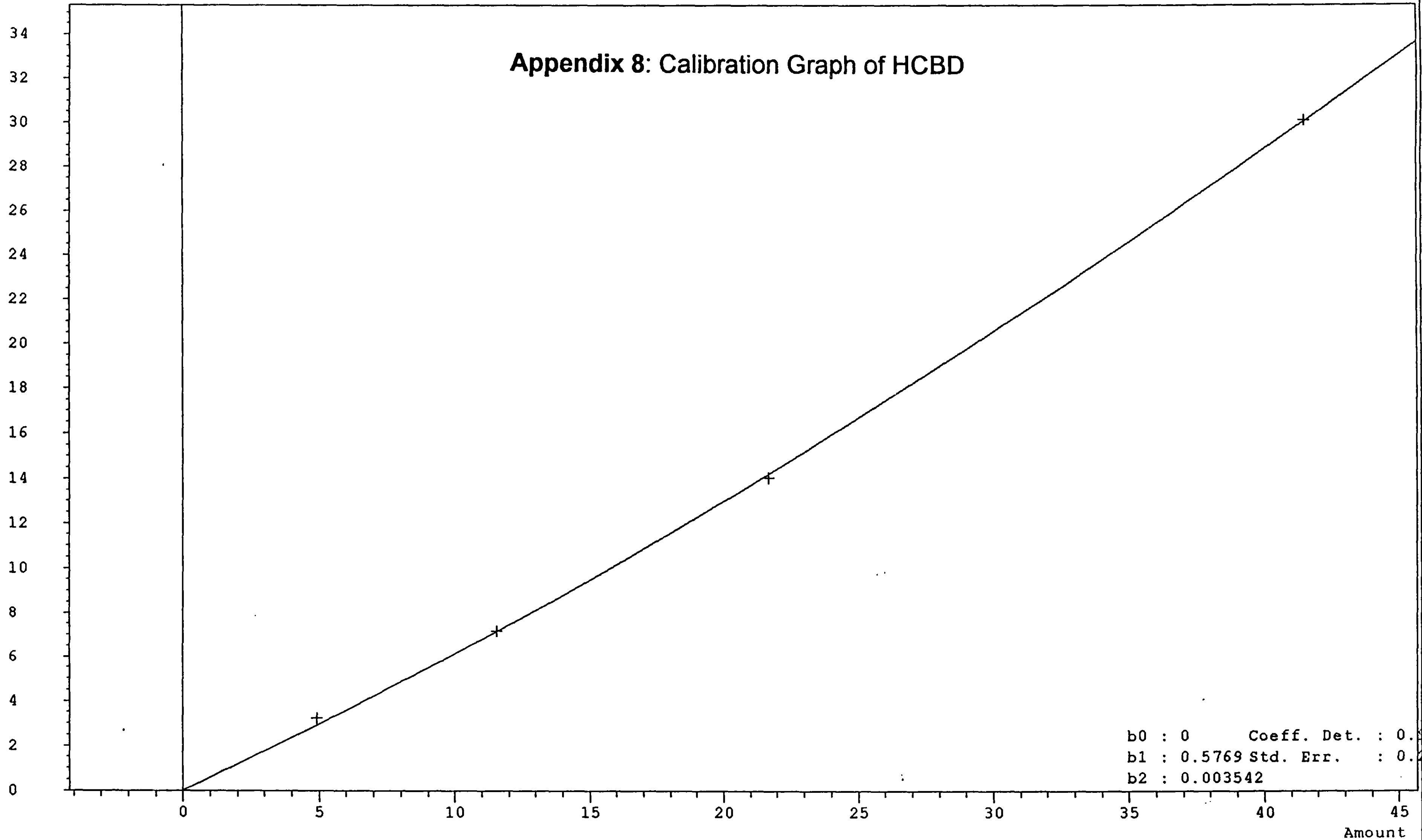
PCB198
Cis Perm
Trans Perm
DCBP
Cypermethrin
Deltamethrin



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