Analysis of Organochloro-Pesticides in Eritrean Water and Sediment Samples.

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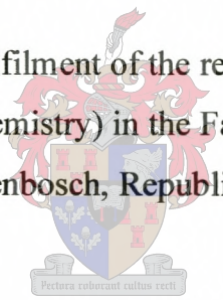
Thesis presented in partial fulfilment of the requirements for the degree of Masters of Science (Chemistry) in the Faculty of Science at the University of Stellenbosch, Republic of South Africa.



Supervisor: Prof. Dr. P. J. F. Sandra

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Summary

The choice of the sample preparation technique used in combination with a chromatographic technique in environmental analysis strongly influences the performance of the method as a whole. The main aim of this work was to evaluate methods like: micro liquid-liquid extraction (μLLE) and solid phase micro extraction (SPME) in combination with gas chromatography-electron capture detector (GC-ECD) and a recently developed technique named stir bar sorptive extraction (SBSE) combined with thermal desorption system-programmable temperature vaporization-gas chromatography-mass spectrometer (TDS-PTV-GC-MS) for the analysis of organochloro-pesticides (OCPs) in natural water and to make a comparison between them. The most suitable method was then selected for the analysis of water samples collected from Eritrea. An additional goal was to develop a sensitive method for the analysis of OCPs in soil and sediment samples.

μLLE-GC-ECD and SPME-GC-ECD methods were evaluated for the analysis of OCPs in water. The former allowed achieving a sensitivity of less than 100 ppt and the latter was able to detect down to the 1 ppt level. Both methods were found to be repeatable with %RSD of 3% to 19% and 5% to 12% as well as linear with a range of $1{:}10^3$ and $1{:}5x10^3$ respectivey. SBSE-TDS-GC-MS was also investigated for the analysis of OCPs in water samples. With this technique a good sensitivity down to 1 ppt was also obtained. The less sensitive nature of the MS compared to ECD was hereby compensated for by the large amount of polydimethylsiloxane (PDMS) phase on the stir bar. The method was repeatable and linear with a range of 1:5000, which is similar to the one obtained for the SPME-GC-ECD method. All three methods were also tested with real contaminated water samples and a comparison of the three techniques in terms of sensitivity, linearity, repeatability, availability and cost effectiveness was done. As a method of choice in terms of the above criteria, SPME-GC-ECD was applied to the water samples collected from Eritrea. The presence of some OCPs such as α-BHC (benzenehexachloride), heptachlor, heptachlor epoxide, endosulfan I, p,p'-DDE (dichlorodiphenylchloroethylene), endosulfan II, p,p'-DDD (dichlorodiphenyldichloroethane), endosulfan sulfate and p,p'-DDT (dichlorodiphenyltrichloroethane) in some of the Eritrean water samples was demonstrated.

The SPME-GC-ECD technique for the analysis OCPs in soil and sediment samples was investigated. It was shown to be able to detect down to 1 pg/g (1ppt) with good linearity and repeatability. The method was also evaluated for authentic soil samples. Due to a lack of time the method could not be applied for the analysis of the soil and sediment samples collected from Eritrea.

Opsomming

Die keuse van 'n gepaste monster-voorbereidingsmetode wat gebruik word tesame met 'n chromatografiese skeidingtegniek in omgewings-analise het 'n beduidende effek op die algehele metode. Die doel van hierdie werk was om metodes soos mikro-vloeistof-vloeistof ekstraksie (μLLE) en soliede fase mikro ekstraksie (SPME) in kombinasie met gaschromatografie met elektronvangs deteksie (GC-ECD) te ontwikkel vir die analise van organochloor-pestisiede (OCPs) in natuurlike water. Vervolgens is hierdie metodes vergelyk met die onlangs-ontwikkelde roerstaaf sorptiewe ekstraksie tegniek (SBSE) in kombinasie met termiese desorbsie-GC-massaspektrometriese deteksie (TDS-GC-MSD). Die mees geskikte metode is vervolgens gebruik vir die analise van Eritreaanse watermonsters. Daarbenewens was die doel ook om 'n sensitiewe metode te ontwikkel vir die analise van OCPs in grond- en sedimentmonsters.

Beide μLLE en SPME-GC-ECD metodes is gekarakteriseer deur goeie herhaalbaarheid en lineariteit. Die sensitiwiteit van bogenoemde metodes was minder as 100 dele per triljoen (ppt) en 1 ppt, respektiewelik. Ook met SBSE-TDS-GC-MSD kon 'n deteksielimiet van 1 ppt bereik word. In hierdie geval is vir die verlies van sensitiwiteit van die MSD in vergelyking met die ECD, gekompenseer deur die groter hoeveelheid PDMS op die roerstaaf in vergelyking met 'n SPME apparaat. Die herhaalbaarheid en lineariteit van die metode is vergelykbaar met dié van die SPME-GC-ECD metode.

Al drie metodes is vergelyk vir die analise van gekontamineerde watermonsters in terme van sensitiwiteit, liniêre bereik, herhaalbaarheid, toeganklikheid en koste-effektiwiteit. SPME-GC-ECD is vervolgens gekies as metode van voorkeur vir die analise van Eritreaanse watermonsters. Die teenwoordigheid van sekere OCPs soos α-BHC, heptachloor, heptachloorepoksied, endosulfan I, p,p'-DDE, endosulfan II, p,p'-DDD, endosulfan sulfaat en p,p'-DDT in die monsters is gedemonstreer.

Die toepasbaarheid van die SPME-GC-ECD metode vir die analise van OCPs in grond-en sedimentmonsters is ook geëvalueer, en dit was moontlik om so min as 1 pg/g waar te neem. Weens tydbeperkings was dit nie moontlik om Eritreaanse grondmonsters met die ontwikkelde metode te analiseer nie.

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List of Abbreviations

Ar/CH4	- Argon/Methane
BHC	- BenzeneHexaChloride
CGC	- Capillary Gas Chromatography
CIS	- Cooled Injection System
DDD	- DichloroDiphenylDichloroethane
DDE	- DichloroDiphenylchloroEthylene
DDT	- DichloroDiphenylTrichloroethane
DMDS	- DiMethylDichloroSilane
ECD	- Electron Capture Detector
EDCs	- Endocrine Disrupting Chemicals
EI	- Electron Impact ionization
EPA	- Environmental Protection Agency
EU	- European Union
EWS	- Eritrean Water Sample
FID	- Flame Ionization Detector
HPLC	- High Performance Liquid Chromatography
I.S.	- Internal Standard
kPa	- kilopascal
LC-UV	-Liquid Chromatography-UltraViolate detector
LD	- Liquid Desorption
LLE	- Liquid-Liquid Extraction
LOD	- Limit Of Detection
LOQ	- Limit Of Quantification
µ	- micro
mg	- milligram
µg	- microgram
ml	- milliliter
µl	- micro liter
µLLE	- Micro Liquid-Liquid Extraction
mm	- millimeter
MS	- Mass Spectrometer

µs	- microsecond
MSD	- Mass Selective Detector
nA	- nanoampere
ng	- nanogram
OCPs	- OrganoChloro-Pesticides
PAH	- PolyAromatic Hydrocarbons
PCBs	- PolyChlorinated Biphenyls
PDMS	- PolyDiMethylSiloxane
pg	- picogram
PP	- Power Plant
ppb	- parts per billion
ppt	- parts per trillion
PTV	- Programmable Temperature Vaporization injector
RSD	- Relative Standard Deviation
SBSE	- Stir Bar Sorptive Extraction
SIM	- Selective Ion Monitoring
SPE	- Solid Phase Extraction
SPME	- Solid Phase Micro Extraction
TDS	- Thermal Desorption System
TDU	- Thermal Desorption Unit
TIC	- Total Ion Chromatogram
UTM	- Universal Transverse Mercator
V	- Volt
WTP	- Water Treatment Plant

Table of contents

General Introduction

Pesticides are by purpose toxic chemicals, which are introduced directly in the environment. They are used in agriculture, homes, and urban areas to control insects, weeds, etc. A particularly worrying class is the organochloro-pesticides (OCPs), which comprises some particularly toxic components. These chemicals are known for their potential to cause birth defects, cancer and other diseases, and to accelerate the rate of degenerative disorders.

Although OCPs were banned years ago, they are still found in developed countries as residues of their past use. Due to lack of sufficient information, their low cost or simply because of the lack of alternatives, OCPs are still used in many third world countries. For example DDT (dichlorodiphenyltrichloroethane) is still applied as the only reliable option for malaria control in Eritrea, Ethiopia, South Africa and other countries. Therefore the development and improvement of affordable analytical methods that permit the simultaneous determination of the main OCPs with minimum extraction and clean-up steps remains an important issue.

Currently gas chromatography (GC) coupled to various detectors is the main tool for trace analysis of both volatile and semi-volatile pesticides with or without prior derivatization. Environmental analysis generally consists of three main steps: sample collection, sample preparation and sample analysis. Sample preparation is often the most difficult and time-consuming step [1]. In the past few years a number of analytical methods for enrichment of OCPs from different sample matrices have been developed. Most commonly used are: liquid-liquid extraction (LLE) [2], solid-phase extraction (SPE) [3], purge and trap [4], static headspace [5], micro liquid-liquid extraction (μLLE) [6], solid phase micro extraction (SPME) [7] and the recently introduced stir bar sorptive extraction (SBSE) [8]. These sample preparation methods are detailed in Chapter II.

SPME and SBSE use a sorbent for the extraction and concentration of analytes from aqueous matrices. In SPME a fiber is coated by a polymeric stationary phase with a volume of 0.5 μl that can be easily extracted and retracted from a protective needle. A major advantage of SPME is that injection can be performed in a conventional

split/splitless injector. Though there are different coatings available, polydimethylsiloxane (PDMS) based materials are preferable because they allow true sorptive extraction of the analytes. SBSE uses stir bars 10 to 40 mm in length, coated with 50 to 220 µl of PDMS. The stir bar is introduced in a specially designed thermal desorption unit (TDU) for injection to the GC. Hence, the difference between SPME and SBSE is found in the amount of PDMS coating used for extraction. The much larger amount of PDMS in SBSE leads to drastically improved recoveries.

The GC separation is generally performed on capillary columns with a PDMS coating (often with 5% phenyl substitution to increase stability and polarity) and detection is done by either electron capture detection (ECD) or mass spectrometry (MS). The former offers very high sensitivity while the latter allows structural elucidation but suffers from reduced sensitivity in the scan mode.

In this study three analytical methods, namely µLLE-GC-ECD, SPME-GC-ECD and SBSE-TDS-GC-MS, are compared for the analysis of OCPs in water samples. The methods have been evaluated in terms of sensitivity, repeatability, reproducibility, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. SPME-GC-ECD was also evaluated for the analysis of OCPs in soil and sediment samples. Finally, SPME in combination with ECD was used for analysis of OCPs in genuine water samples collected in Eritrea.

References

1. J. Pawliszyn, "Solid Phase Microextraction: Theory and Practice", Wiley, New York, USA, **1997**.
2. O.S. Fatoki and R.O. Awofolu. *J. Chromatogr. A*, 2003, 983, 225–236.
3. A.R. Fernandez-Alba, A. Aguera , M. Contreras , G. Penuela , I. Ferrer and D. Barcelo. *J. Chromatogr. A*, **1998**, 823, 35–47.
4. M. Mezcua, A. Aguera, M. D. Hernando, L. Piedra and A. R. Fernandez-Alba. *J. Chromatogr. A*, **2003**, 999, 81–90.
5. T. Wenzl and E. P. Lankmayr. *J. Chromatogr. A*, **2000**, 897, 269–277.
6. A. Zapf, R. Heyer and H. Stan. *J. Chromatogr. A*, **1995**, 694, 453-461.
7. C. L. Arthur, J. Pawliszyn. *Anal. Chem.* **1990**, 62, 2145.
8. E. Baltussen, P. Sandra, F. David and C. Cramers. *J. Microcol. Sep.* **1999**, 11(10), 737-747.

Chapter I

Endocrine disrupting chemicals and their relevance in nature

The safety of our environment is currently of major concern all over the world. As the number of toxic and hazardous chemicals increases, the public awareness is also increasing. Much evidence has been accumulating in the last decades indicating that humans, domestic and wildlife species suffer from exposure to environmentally hazardous chemicals that interact with the hormonal system [1]. Theses chemicals are often called endocrine disrupting chemicals (EDCs) [2]. They interfere with the human and animal metabolism, reproduction, and other biological activities in the body by mimicking or by binding to the active site of the hormonal receptors [3,4]. The main classes of compounds corresponding to the above-mentioned pollutants are: pesticides (chlorinated, nitrogenated and phosphorated organic compounds), polychlorinated biphenyls (PCBs), dioxins, synthetic and natural estrogens, alkylphenols, phthalates and organometallic compounds. The emphasis in this work is placed on the chlorinated pesticides.

Pesticides are routinely used in agriculture as a way of controlling insects, plant diseases, worms, and others [5]. As these pesticides are released to the environment, they can easily be leached to the water body (which can be either surface or ground water) or remain in soil for a long time [6]. From the roughly 1 million known species of insects, 10,000 of them are crop-eating and 700 of these insects are considered as harmful for crops throughout the world. The use of pesticides has been known since 1000 B.C. One of the earliest pesticides was brimstone or natural sulfur, which was used as a means of insect control. Afterwards many materials like tobacco, soapy water, whitewash, vinegar, fish oil, etc. have been applied with varying success. Only after World War II insect control by chemical-synthetic organic insecticides was introduced. DDT (dichlorodiphenyltrichloroethane) was discovered in 1939 by Paul Mueller and was first registered in Canada for insect control in 1946. It was widely used until the 1980's when it was banned together with a series of other OCPs in most countries [7].

Many toxic pesticides are still present in our environment due to the high demand for pesticides in the past. The presently used pesticides are less toxic and short-living compounds [8]. At present there are about 800 pesticides and 600 (herbicides, fungicides, insecticides, nematicides, growth regulators, synergists, etc.) are intensively used and are listed in various pesticides manuals [9]. The pesticides selected for this study were the group of chlorinated pesticides, banned years ago, of which residues are still found in the environment.

Organochloro-pesticides (OCPs) are insecticides composed of carbon, hydrogen and chlorine. These pesticides are well-known for their toxicity towards human and animal life and especially in their disrupting effect on the endocrine system and growth hormones [10]. Therefore it was crucial to control the spread and use of these compounds. As the public awareness about the damage done to the environment increased over time, the European Union (EU), the Environmental Protection Agency (EPA, USA) as well as associated organizations set rules and regulations for pesticide use and compiled lists of pesticides to be banned. The OCPs are of historic interest these days because only few of them exist in today's arsenal [7]. The main aim of these regulatory agencies is to eradicate the use of persistent chemicals like DDT and to encourage the development of secure, target-specific compounds [8]. Although DDT saved millions of lives by killing the malaria mosquito, it never achieved complete success in the world's poorer countries. Following complaints from environmentalists in the 1970s, DDT was removed from the malaria control program in many developing countries, but continued to be used in more than 20 countries, most of them in Africa, like Eritrea, Ethiopia, South Africa and others, for fighting malaria as the only viable option.

The OCPs can be classified into three groups [11,12]:

1. Hexachlorocyclohexanes (α-BHC, β-BHC, γ-BHC and δ-BHC),
2. Cyclodienes (aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide, endosulfan I, endosulfan II and endosulfan sulfate) and
3. Diphenyl aliphatics (p,p'-DDT, p,p'-DDD, p,p'-DDE and methoxychlor)

The chemical structures are given in table 1.1, 1.2, and 1.3, respectively.

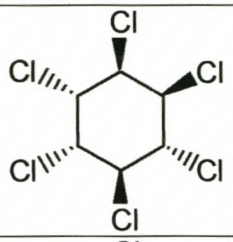
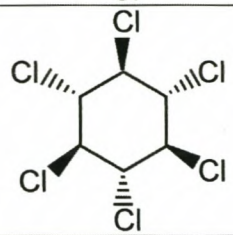
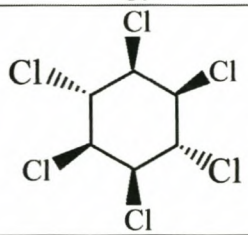
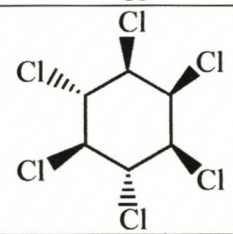
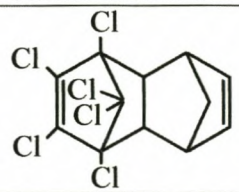
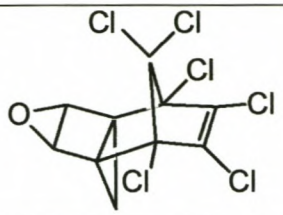
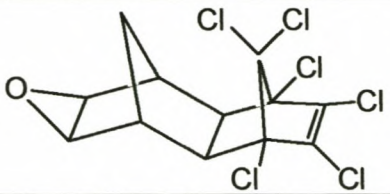
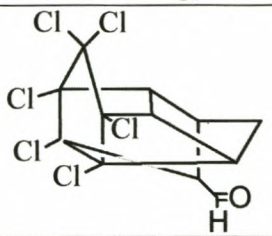
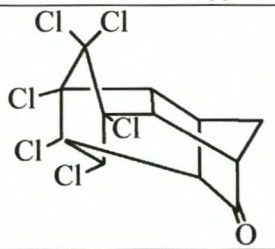
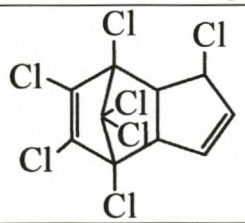
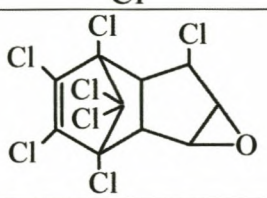
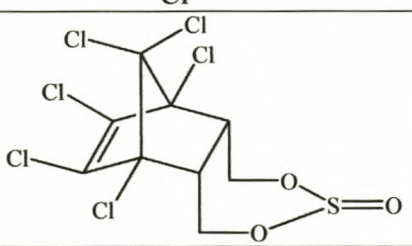
Compound name	Chemical structure
α-BHC	
β-BHC	
γ-BHC	
δ-BHC	

Table 1.1. Structural representation of the hexachlorocyclohexane group.

Compound name	Chemical structure
Aldrin	

Dieldrin	
Endrin	
Endrin aldehyde	
Endrin ketone	
Heptachlor	
Heptachlor epoxide	
Endosulfan I	

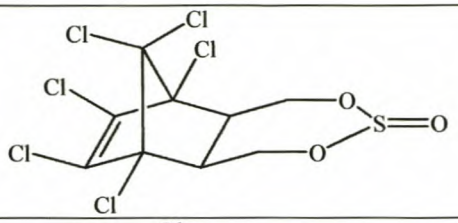
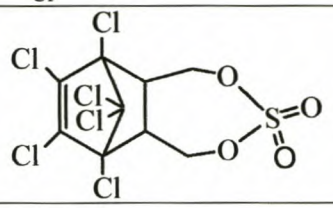
Endosulfan II	
Endosulfan Sulfate	

Table 1.2. Structural representation of the cyclodiene group.

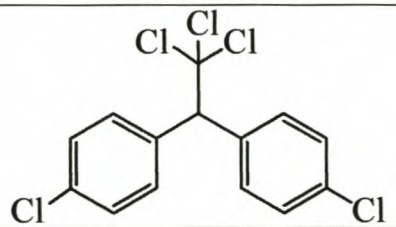
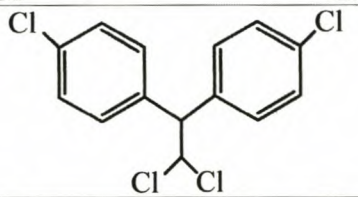
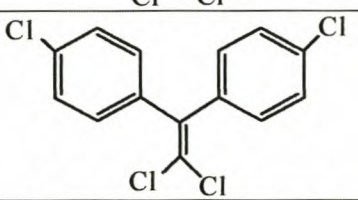
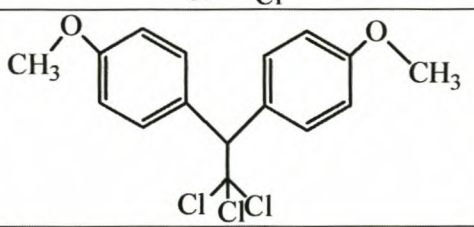
Compound name	Chemical structure
p,p'-DDT	
p,p'-DDD	
p,p'-DDE	
Methoxychlor	

Table 1.3. Structural representation of the diphenyl aliphatic group.

Eritrea, one of the developing countries in Africa, is located in the Sahelian zone of Africa and has a total area of 124,000 square kilometres. Its geographical co-ordinates are N 12° 18" latitude and E 36°43" longitude. Being an arid and semi-arid country it is not gifted with rich water resources. It has 3.5 to 4.0 million people and 80% of them are dependent on agriculture. The main rainy seasons are July and August and the rainfall during these months is torrential, of high intensity and spotty in nature.

The capital city, Asmara, is located in the semi-arid zone at 2300 m above sea level and about 115 km off the Red Sea coast. Except the capital and surrounding villages, the rest of the country depends on groundwater for surviving [13,14]. The area selected for this study was the capital and surrounding villages called the Central zone (zoba Ma'akel). This area was selected for two reasons: almost all of the area depends on surface water (lakes or dams) and it is the main industrialized area and consequently more polluted compared to the rest of the country. Although the area selected shows a lower rate of malaria compared to the rest of the country, DDT is still used as tool to control malaria and as an insecticide in some households [15].

Because OCPs are so harmful and are still found in vast amounts in the environment, it is vital to develop sensitive, selective, accurate, precise, cheap, and applicable analytical methods for their analysis. Due to the requirement to quantify them in trace and ultra-trace levels in complex matrices like soils and water, state-of-the-art gas chromatographic techniques are demanded [2,8]. The different sample preparation methods and types of gas chromatography (GC) instruments are detailed in chapter II and III.

References

1. F. Regan, A Moran, B. Fogarty and E. Dempsey. *J. of Chromatogr. B*, **2002**, 770, 243-253.
2. M. Morita. International Symposium on *Coastal Hydrosphere*, Tokyo, Japan (Feb. 9-10, **1999**).
3. A. Peñalver, V. García, E. Pocurull, F. Borull, and R.M. Marcé. *J. Chromatogr. A*, **2003**, 1007, 1-9.
4. T. Benijts, J. Versammen, R. Dams, H. P. Tuan, W. Lambert, and P. Sandra. *J. Chromatogr. B*, **2001**, 755, 137-142.
5. S. Babić, M. Petrović and M. K. Telan-Macan. *J. Chromatogr. A*, **1998**, 823, 3-9.
6. J. L. Martínez Vidal, F. J. Arrebola and M. Mateu-Sánchez. *J. Chromatogr. A*, **2002**, 959, 203-213.
7. G. W. Ware. *An Introduction to Insecticides, 3rd edition*, **08 Apr. 2003**, 1-11. (http://ipmworld.umn.edu/chapters/ware.htm).
8. K. Attra. Buck Scientific, *Application Note #GC3006*.
9. C.D.S. Tomlin, Editor, *The Pesticide Manual 12th ed.*, The British Crop Protection Council, Farnham, Surrey (**2000**).
10. J. C. R. Demyttenare, J. I. S. Martínez, R. Verhé, P. Sandra and N. De Kimpe. *J. Chromatogr. A*, **2003**, 985, 221-232.
11. J. C. R. Demyttenare, J. I. S. Martínez, R. Verhé, P. Sandra and N. De Kimpe. *J. Chromatogr. A*, **2003**, 985, 221-232.
12. R. Doong and P. Liao. *J. Chromatogr. A*, **2001**, 981, 177-188.
13. *Review on Profile of Water Supply Situation in Asmara*, Water Resources Department, Asmara, Eritrea, **1995**.
14. H. Zerai. *Review on Evaluation of Ground Water Potential of Asmara and its Surroundings*. Eritrean Water Resources Department, **1995**.
15. Government of Eritrea. *National Environmental Management Plan for Eritrea*, Drukerei Schwenk and Co. GmbH, Frankfurt, Germany, **1996**.

Chapter II

Sample preparation techniques for organochloro-pesticides analysis

2.1. Introduction

In the last few years several new sample preparation techniques have been developed which are of potential interest for environmental analysis [1]. In most of the environmental samples the concentration of the analytes to be detected is very low and often below the detection limit of the instrumentation. Therefore sample extraction and enrichment is an important part of trace and ultra-trace analysis [2]. In this chapter an overview of the most common sample preparation (extraction and enrichment) methods relevant to environmental analyses are presented.

2.2. Liquid-liquid extraction (LLE)

Liquid-liquid extraction is a classical method, which is used for the extraction of analytes of concern from aqueous matrices [1]. LLE is also called separation funnel extraction [3]. The aqueous sample solution is mixed with an organic solvent and shaked manually or mechanically. LLE is loosing favor because the clean-up procedure is time consuming and can result in loss of analyte. Moreover, it requires high labor costs and uses large amounts of high purity often toxic organic solvents. The load of the latter to the environment often outweighs the benefits for OCP analysis [1,4,5]. Nevertheless, LLE has extensively been used in the past for this purpose [6-8]. In some cases LLE can also be used for the extraction of highly polar pesticides [9].

2.3. Micro liquid-liquid extraction (μLLE)

To overcome some of the above-mentioned drawbacks of LLE, miniaturization has become a main trend in sample preparation. μLLE is a typical example [10]. In μLLE the same is done as in LLE except that smaller solvent and sample volumes are used. It is also called in-vial-extraction. In μLLE less than 1 ml of organic solvent is used for the extraction [5,11]. Subsequently 1 μl of the organic phase is generally injected

in the GC for analysis. In this study 200 µl of organic solvent was used. Not surprisingly µLLE of OCPs has been reported as the better option compared to LLE [12].

2.4. Solid phase extraction (SPE)

Solid phase extraction is based on partition, adsorption, affinity or ion exchange mechanisms and is faster than the classical LLE methods. The principle of retention is analogous to those that are used in high performance liquid chromatography (HPLC). It is suitable for low, intermediate and high polarity pollutants, depending on the sorbent phase used. Large sample volumes can be handled using relatively small amounts of solid phase, which in turn requires small volumes of solvent for the solid phase stripping, eliminating the need for an additional evaporation step and considerably reducing the risk of contamination. Depending on the sample throughput and the compounds to be analyzed, the extraction may be performed either on a cartridge or on membrane discs [1,13].

In most of the applications, SPE uses cartridges containing 100 mg to 1 g of a non-polar phase. In principle the cartridges are not reusable. As an alternative to the standard SPE cartridges, extraction discs (46 mm diameter) were introduced several years ago allowing higher sampling flow rates (1 litre in 10 min.) and reduced drying times [13]. SPE uses smaller amounts of organic solvent (but not negligible) compared to LLE. The combination of SPE with GC is a good alternative for the analysis of OCPs from aqueous matrices [8,14,15].

2.5. Membrane extraction

These techniques can provide some characteristic advantages over the techniques mentioned above (LLE and SPE), especially regarding selectivity, enrichment power and automation potential.

Membrane extraction techniques can be divided into two main categories, porous and non-porous membrane techniques. Another classification is between two-, or three-phase membrane extraction techniques. In all types of membrane extraction, the

membrane separates the sample solution (called donor or feed solution) from the acceptor or strip solution and the analyte molecules pass through the membrane from the donor to the acceptor. This process is sometimes called pertraction (permeation-extraction) [16].

The aim is to transfer as much of the analyte as possible from the donor to the acceptor phase. To improve this recovery, the acceptor phase is in many cases flowing, so that extracted analytes are removed from the membrane by convection. In some cases, the analytes can be trapped in the acceptor either by a chemical reaction or simply because of a high partition coefficient and this will lead to high enrichment factors. To improve the overall extracted amount of analyte, a flowing donor is also used, the sample being pumped on the donor side of the membrane. Some applications of membrane techniques for sample preparation coupled to GC-MS for OCPs [17] and LC-UV for herbicides [17], have been reported.

2.6. Purge and trap (gas phase extraction)

Dynamic gas phase extraction of aqueous samples, called purge and trap is generally applicable for the extraction of volatile organic compounds out of aqueous solutions. An inert gas is bubbled through the water sample, causing the purgeable organics to move from the aqueous to the vapor phase. The volatile compounds are then trapped on an adsorbent such as Tenax or activated charcoal. The trap containing the sorbent material is generally built in a desorption chamber equipped with a heating device, which when activated permits the desorption of the trapped compounds. This technique has the distinct merit of providing a very clean sample, free from its often very dirty matrix [1,13].

The technique is used routinely in many laboratories for the analysis of highly volatile organic compounds in environmental samples such as sea-water [18] drinking water [19] and soil [20]. The technique is less successful in enriching semi-volatile OCPs.

2.7. Static and dynamic headspace extraction

Headspace analysis is generally defined as a vapor-phase extraction, involving the partitioning of analytes between a non-volatile liquid or solid phase and the vapor phase above the liquid or solid [21].

Headspace analysis is used to analyse volatiles whose matrix is of no interest, for example water, soil, polymers etc. The various commercially available headspace auto-samplers are based on the principle of static or dynamic headspace extraction.

In static headspace, a water sample is transferred to a headspace vial, sealed and placed in a thermostat to drive the volatile components into the headspace for sampling. An aliquot of the vapour phase is introduced via a gas-tight syringe or a sample loop of a gas-sampling valve into the GC. Static headspace implies that the sample is taken from one phase in equilibrium [1]. Static headspace sampling, because of its limited sensitivity, is mostly employed for applications in the ppm level to percent concentration ranges [21]. Headspace extraction for volatile aldehydes and aromatic compounds under these conditions have been reported [22].

Dynamic headspace extraction uses a continues flow of gas for removal of headspace vapors above liquid or solid samples and subsequent collection of the compounds of interest. It is used for the determination of analytes at low concentration or compounds that have unfavorable partition coefficients for their analysis in the static headspace method [3,5].
Both techniques are not successful for the enrichment of OCPs.

2.8. Solid phase micro extraction (SPME)

Nowadays extraction with large quantities of toxic solvents for the determination of pesticides is considered environmentally unfriendly and solventless sample preparation techniques are preferred [23]. Therefore and because of the need for fast, simple and cheap analytical extraction methods, a completely solvent free extraction method called solid phase micro extraction (SPME) was introduced in the 90's by Pawliszyn and co-workers [24]. This technique uses a fused silica fiber coated with, for example, a polymeric stationary phase for extraction from which the analytes are then subsequently thermally desorbed in a standard split/splitless GC injector for analysis. SPME integrates extraction and concentration in a single step. This method provides a significantly more rapid, simple and easy to perform extraction compared to the traditional extraction techniques [6-8].

Various phases (coatings) are available: Polydimethylsiloxane (PDMS), Polyacrylate (PA), Carbowax-Divinylbenzene (CW-DVB), Polydimethylsiloxane-Divinylbenzene (PDMS-DVB), Polydimethylsiloxane-Carboxen (PDMS-CAR), and Polydimethylsiloxane-Carboxen-Divinylbenzene (PDMS-CAR-DVB) combinations. The choice of a particular coating depends on the chemical structure of the compounds of interest. As a general selection rule, the "like dissolves like" principle can be applied. Hence, the selectivity is based on polarity and volatility differences between molecules [24-26]. The development of the technique rapidly accelerated with the implementation of coated fibers incorporated into a microsyringe, resulting in the first commercial SPME device. Fig. 2.1. shows one of the early designs of this device based on the Hamilton E 7000 series microsyringe [24].

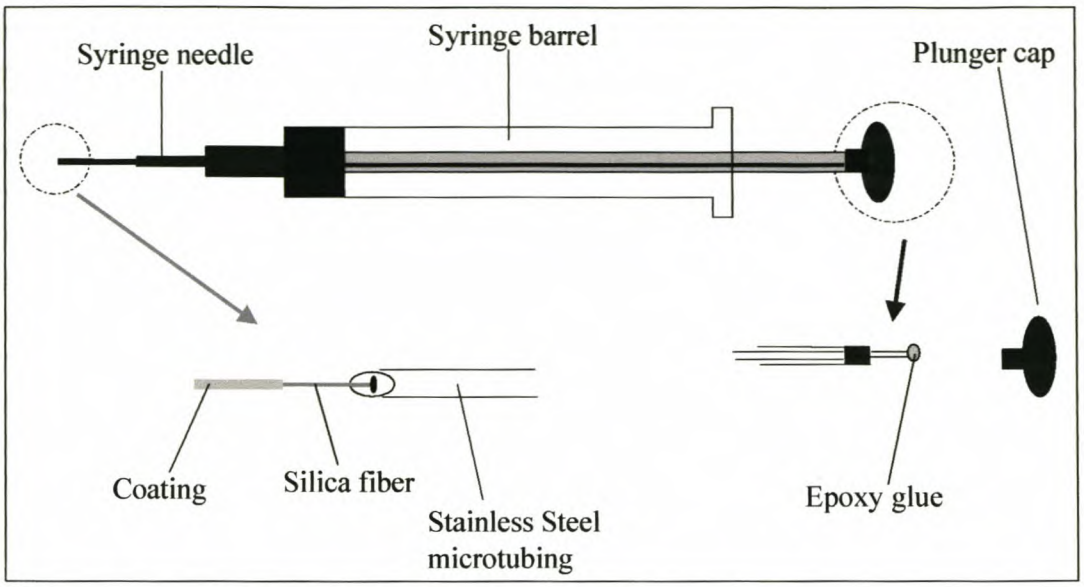


Fig. 2.1. Custom-made SPME device based on a Hamilton 7000 series syringe.

There are three sampling modes in SPME: direct extraction, headspace extraction, and membrane protected SPME. In direct extraction, the coated fiber is directly immersed in the sample and the analytes are distributed between the sample matrix and the fiber coating. In order to speed up the process, agitation is necessary. In the headspace mode the analytes need to be transported through the vapour phase above the liquid before they can reach the coating. This mode of extraction protects the fiber coating from damage by high-molecular-mass interferences such as humic matter. The membrane protected SPME is used for the extraction of compounds from highly polluted samples in order to protect the fiber from damage. Membrane protection is advantageous for determination of analytes having volatilities too low for the headspace approach. Moreover, a membrane made from the appropriate material can be used for selective extraction of target compounds [24,25]. The three modes of SPME are shown in Fig 2.2. [24].

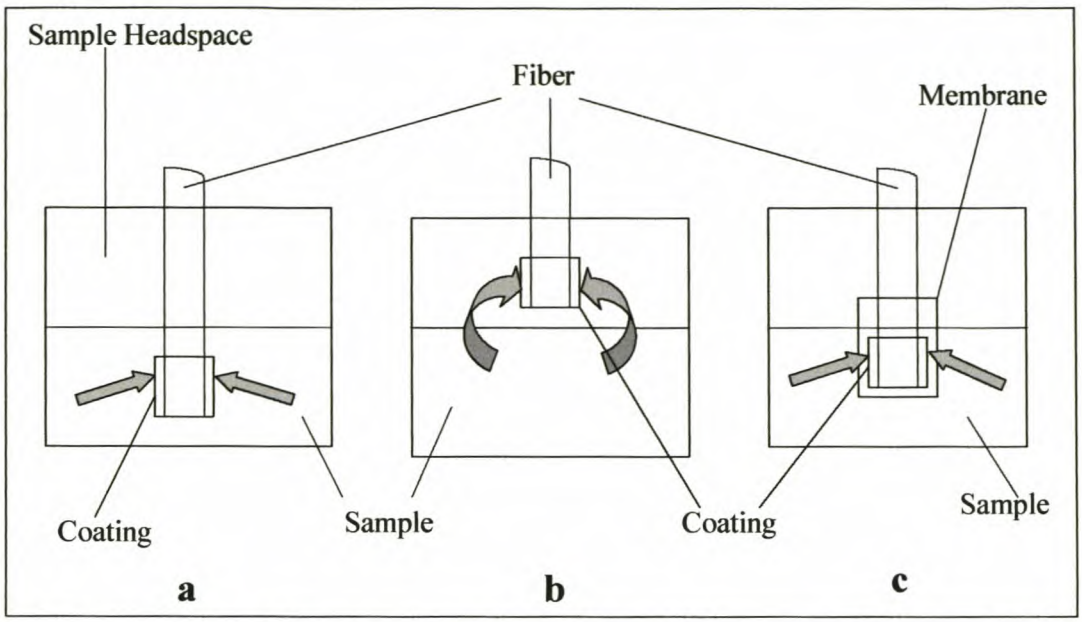


Fig. 2.2. Modes of SPME operation: (a) direct SPME, (b) headspace SPME, (c) membrane-protected SPME.

The thermodynamic aspects of SPME have been comprehensively studied and show that the amount of analyte extracted by the coating is directly proportional to the analyte concentration in the sample, the thickness of the polymer coating and the distribution constant for the analytes [27]. *In the case of PDMS or PA coatings, the extraction process is called sorptive extraction which involves the analytes being extracted from the matrix (mostly aqueous) into a non-miscible pseudo-liquid phase* [1,5,10]. *On the other materials adsorptive extraction takes place, i.e. the analytes are bound to sites on the surface.* The total amount of extracting phase (volume) is important in sorptive extraction [2]. The amount of material extracted is independent of the fiber positioning. It can be placed in the headspace or directly in the sample. Moreover the amount of analytes absorbed by the fiber is dependent on the extraction time. The latter should be optimised to obtain extraction of the analytes of interest in a reasonable amount of time [10,28]. Extraction can indeed be performed under equilibrium or non-equilibrium conditions. In the first mode the analyte concentration reaches equilibrium between the sample matrix and the fiber coating. Hence the amount extracted remains constant within the limits of experimental error and it is independent of further increase in extraction time [24,25,27]. Under equilibrium conditions the following equation is valid:

$$n = \frac{K_{fs} V_f V_s C_o}{K_{fs} V_f + V_s} \qquad (2.8.1)$$

where n is the number of moles extracted by the coating, K_{fs} is the concentration of analyte in the fiber coating divided by concentration of analyte in sample matrix, V_f is the fiber coating volume, V_s is sample volume and C_o is the initial concentration of the analyte in the sample.

When the sample volume is very large ($K_{fs}V_f << V_s$), equation (2.8.1) can be reduced to:

$$n = K_{fs} V_f C_o \qquad (2.8.2)$$

which shows the advantage of this method for field application. In this case, the amount extracted is independent of the sample volume.

When sampling time is too long to reach equilibrium, SPME is performed in the non-equilibrium mode. In this mode the sampling time must be carefully controlled.

The sensitivity of SPME can be enhanced by:

- Increasing the volume of the fiber coating.
- Using a selective coating for target analytes to increase K_{fs}.
- Optimising the temperature thereby changing the K_{fs}.

An increase in temperature enhances the sensitivity for high boiling compounds but reduces for lower boiling compounds. For highly volatile compounds temperature plays the opposite role, i.e. as the temperature increases the sensitivity decreases due to a decrease of K_{fs}. Increasing the sample temperature increases diffusion coefficients and decreases distribution constants, which both lead to a faster equilibrium. In this way the kinetics of SPME determine the speed of extraction [24,27].

SPME has been successfully applied for the analysis of a wide range of highly volatile to almost non-volatile organics in combination with either GC or HPLC. Some of the multitudes of applications are the analysis of polychlorinated biphenyls (PCBs) [29], polycyclic aromatic hydrocarbons (PAHs) [30,31], pesticides [32,33], phthalates [34], volatile fatty acids [35], steroids [36,37] and alkyl phenols [36]. Moreover SPME

methods have been successfully applied for the determination of OCPs in water [28,38], soil [32,33,39], biological fluids [40], and in animal tissue [41]. The common most often used polymeric phase is polydimethylsiloxane (PDMS), which is non-polar (Fig 2.3.) and which was also used in this work.

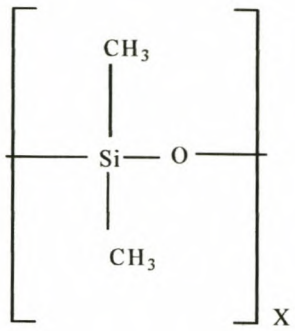


Fig 2.3. Structure of PDMS

Recent studies have correlated this equilibrium constant with octanol/water distribution coefficients ($K_{(O/W)}$). It has been demonstrated that for solutes with low $K_{(O/W)}$ ($k<1000$) low recoveries are obtained [30]. This is mainly due to the phase ratio between the aqueous and PDMS phase. The amount of PDMS used in SPME is typically in the order of 0.5 µl or less, thereby limiting the enrichment on the PDMS fiber [2,10]. Based on these observations, a new approach using stir bars coated with larger amounts of PDMS, called stir bar sorptive extraction (SBSE) was developed [30].

2.9. Stir bar sorptive extraction (SBSE)

Recently, Sandra et al. introduced a new extraction technique based on the same extraction principles as SPME but in this case the sorbent, which varies between 50 to 220 µl PDMS, is placed on a stir bar with dimensions ranging from 10 mm x 3.2 mm to 40 mm x 3.2 mm [13,23,30]. This technique is known as stir bar sorptive extraction (SBSE) and the coated stir bars have been commercialised under the name of Twister® [42,43].

The stir bars have three main parts. The first and inner core part is a magnetic stirring rod that helps transferring the rotating movement from a stirring plate to the sample liquid. The second part of the stir bar is a thin glass jacket that covers the magnetic stirring rod. The third and outer part is the PDMS sorbent layer into which the analytes are extracted (Fig 2.4.) [2].

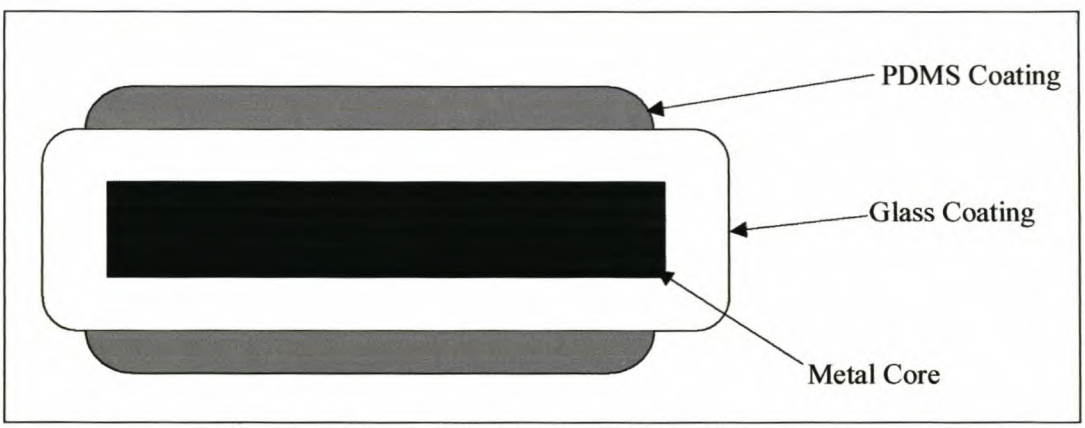


Fig 2.4. Graphic representation of the stir bar.

Hence, the extracting phases are the same as the ones used for SPME. The two techniques only differ in the fact that the amount of coating is up to ca. 500 times larger in SBSE [2,45] and that desorption of the analytes from the stir bar requires a specially designed thermal desorption unit (TDU) mounted on the GC. Liquid desorption can also be used to desorb the compounds from the stir bar [2,44].

As mentioned, sorptive extraction is an equilibrium process, and for water samples the extraction of solutes into the extracting medium is controlled by the partitioning coefficient of the solutes between the silicon phase and the aqueous phase. For PDMS, this partitioning coefficient has been correlated with the octanol-water distribution coefficients ($K_{O/W}$) [1,30]. Even though not fully correct, the octanol-water distribution coefficient gives a good indication if and how well a given solute can be extracted with SPME and SBSE [2,13,30].

The theory of SBSE is similar to that of SPME [25]. With the approximation that the partition coefficients between PDMS and water ($K_{PDMS/W}$) are proportional to octanol-water partition coefficients ($K_{O/W}$), they can be described as [2,13,30]:

$$K_{O/W} \approx K_{PDMS/W} = \frac{C_{SBSE}}{C_W} = \frac{m_{SBSE}}{m_W} \times \frac{V_W}{V_{SBSE}} = \beta \times \frac{m_{PDMS}}{m_W} \qquad (2.9.1)$$

where C_{SBSE} and C_W correspond to the analyte concentration in the stir bar and water phase, respectively; m_{SBSE} and m_W are the mass of analyte in the stir bar and water phase, respectively; V_{SBSE} and V_W correspond to the volume of the stir bar coating and the water phase, respectively, and β is the phase ratio, which is equal to V_W/V_{SBSE}. Equation 2.9.1 can be rewritten as:

$$\frac{K_{O/W}}{\beta} = \frac{m_{SBSE}}{m_W} = \frac{m_{SBSE}}{m_o - m_{SBSE}} \qquad (2.9.2)$$

where m_o is the total mass of the analyte originally present in the water sample. The extraction efficiency or recovery is expressed as the ratio of the extracted amount of analyte (m_{PDMS}) to the initial amount of analyte in the water ($m_o = m_w + m_{PDMS}$). Thus recovery can be calculated by rearrangement of equation 2.9.2 resulting in equation 2.9.3:

$$\frac{m_{SBSE}}{m_o} = \frac{\left(\frac{K_{O/W}}{\beta}\right)}{1+\left(\frac{K_{O/W}}{\beta}\right)} \qquad (2.9.3)$$

Hence, the theoretical recovery for a solute can be calculated if the partition coefficient and the phase ratio are known. Fig 2.5. shows the extraction recovery of an analyte as a function of the $K_{O/W}/\beta$ ratio. At $K_{O/W}/\beta = 1$ the recovery is 0.5 (50%). At low $K_{O/W}/\beta$ values the recovery is approximately proportional to $K_{O/W}/\beta$ whereas at $K_{O/W}/\beta$ values greater than 5 the recovery is almost 1 (100%).

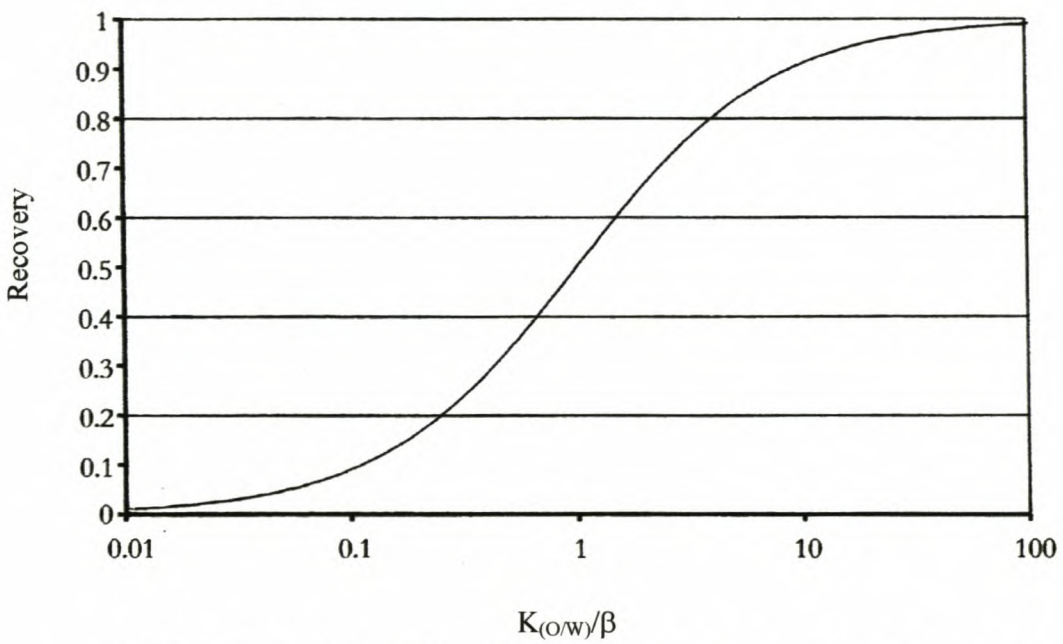


Fig 2.5. Recovery for both SBSE and SPME as a function of the ratio of octanol-water partition constant and phase ratio ($K_{O/W}/\beta$).

In the case of SPME, the volume of the PDMS is approximately 0.5 µl, which results in poor recoveries for analytes with low $K_{O/W}$ values; for example less than 10,000. Since much more PDMS coating (50 to 220 µl) is used in SBSE, the sensitivity increases in a proportional way. The theoretical extraction efficiency reaches 100% for analytes with $K_{O/W}$ values larger than 500 (log P greater than 2.7). For a known sample volume, selected stir bar dimensions and a given analyte, the theoretical

recoveries can be calculated using the K_{OW}WIN software program (Syracuse Research Corp., Syracuse, New York), which is based on a log $K_{O/W}$ calculator.

For a 10 ml water sample a 100 µl of PDMS coated stir bar can be used to get a phase ratio (β) of 100, implying that analytes with a $K_{O/W}$ in excess of 500 are extracted quantitatively into the PDMS coated stir bar. This is not only making quantification easy, but also guarantees a significantly increased sensitivity compared to SPME. Fig 2.6. shows the extraction recovery of compounds from a 10 ml water sample for SPME and SBSE.

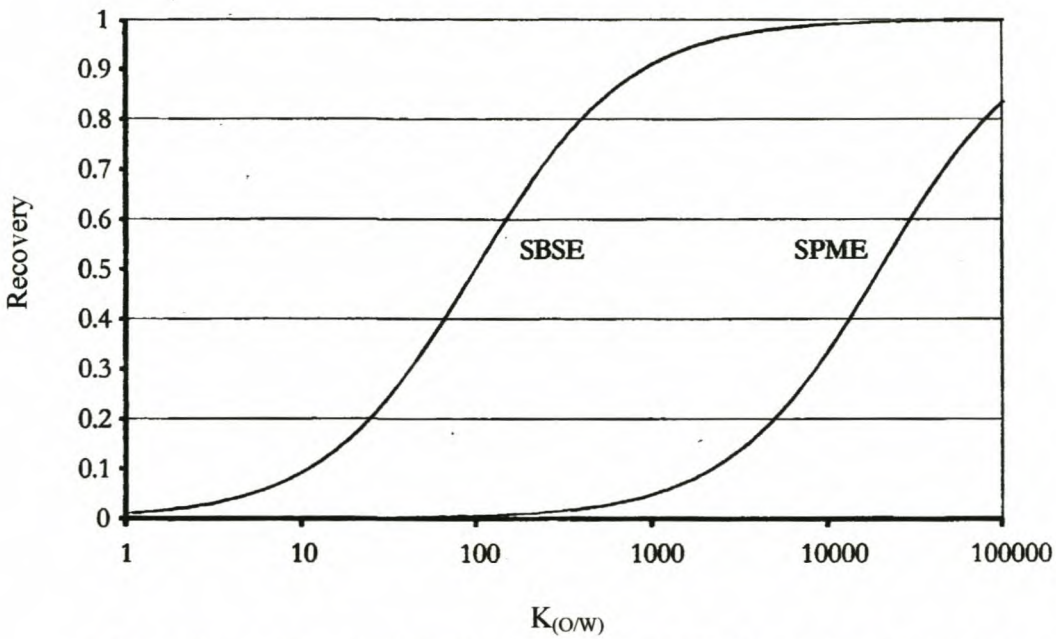


Fig 2.6. Theoretical recovery of analytes using SPME and SBSE for a 10 ml water sample as a function of $K_{O/W}$. Volume of PDMS in SPME: 0.5 µl, and in SBSE stir bar: 100 µl.

It can be seen clearly from Fig 2.6. that quantitative extraction is obtained at a much lower $K_{O/W}$ in SBSE compared to SPME, which is only due to the much lower phase ratio.

Although SBSE is a young technique, many applications have already been described, such as the analysis of OCPs [1,42] and PAHs [2,46,47] in water samples, OCPs in wine [43], carbonyl compounds in beer [48], PCBs in biological fluids [49], volatiles in whisky [50], pesticides in fruits, vegetables, and baby food [23,44] and 2-methylisoborneol and geosmin in water [45].

Reference

1. P. Sandra, R. Soniassy and C. Schlett. "*Water Analysis*", University of Stellenbosch, South Africa, **1998**.
2. F. David, B. Tienpont, P. Sandra and R. E. Majors. *LC-GC North America*, **2003**, 21(2), 1-7.
3. A. G. J. Tredoux, P. J. F. Sandra and H. H. Lauer. *Stir Bar Sorptive Extraction. A Novel Technique to Monitor Micro-pollutants in Aqueous samples*. MSc. Thesis, University of Stellenbosch, South Africa, **2000.**
4. M. Biziuk, A. Przyjazny, J. Czerwinski and M. Wiergowski. *J. Chromatogr. A*, **1996**, 754, 103-123.
5. C. F. Poole and S. K. Poole. *Chromatography Today*, Elsevier, **1999**, The Netherlands.
6. O.S. Fatoki and R.O. Awofolu. *J. Chromatogr. A*, **2003**, 983, 225–236.
7. P. Vinas, N. Campillo, I. Lopez-Garcýa, N. Aguinaga, and M. Hernandez-Cordoba. *J.Chromatogr. A*, **2002**, 978, 249-256.
8. A.R. Fernandez-Alba, A. Aguera , M. Contreras , G. Penuela , I. Ferrer and D. Barcelo. *J. Chromatogr. A*, **1998**, 823, 35–47.
9. N.M. Brito , S. Navickiene , L. Polese , E.F.G. Jardim , R.B. Abakerli and M.L. Ribeiro. *J. Chromatogr. A*, **2002**, 957, 201–209.
10. P. Sandra, E. Baltussen, F. David, and A. Hoffmann. Gerstel *AppNote 1/2000*, **2000**.
11. R. Soniassy, P. Sandra, and C. Schlett, "*Water Analysis*," Hewlett-Packard, Publication (23) 5962-6216E, **1997**.
12. A. Zapf, R. Heyer and H. Stan. *J. Chromatogr. A*, **1995**, 694, 453-461.
13. H.A. Baltussen. *New Concepts of Sorption Based Sample Preparation for Chromatography*. PhD Dissertation, Eindhoven University of Technology, The Netherlands, **2000**.
14. A. Pauwels, D.A. Wells , A. Covaci and P.J.C. Schepens. *J. Chromatogr. B*, **1999**, 723, 117-125.
15. I. Vassilakis , D. Tsipi and M. Scoullos. *J. Chromatogr. A*, **1998**, 823, 49-58.
16. J. Jônsson and L. Mathiasson. *J. Sep. Sci.*, **2001**, 24, 495-507.
17. C. Basheer , H. K. Lee and J. P. Obbard. *J. Chromatogr. A*, **2002**, 968, 191-199.

18. M. Mezcua, A. Aguera, M. D. Hernando, L. Piedra and A. R. Fernandez-Alba. *J. Chromatogr. A*, **2003**, 999, 81–90.
19. E. Martýnez , S. Lacorte , I. Llobet , P. Viana , and D. Barcelo. *J. Chromatogr. A*, **2002**, 959, 181–190.
20. O. Zuloaga, N. Etxebarria, L. A. Fernández and J. M. Madariaga. *Anal. Chim. Acta*, **2000**, 416, 43–53.
21. N. H. Snow. *Trends in Analytical Chemistry*, **2002**, 21, 608-617.
22. T. Wenzl and E. P. Lankmayr. *J. Chromatogr. A*, **2000**, 897, 269–277.
23. P. Sandra, B. Tienpont and F. David. *J. Chromatogr. A*, **2003**, 1000, 299-309.
24. H. Lord and J. Pawliszyn. *J. Chromatogr. A*, **2000**, 885, 153–193.
25. M. de Fátima Alpendurada. *J. Chromatogr. A*, **2000**, 889, 3-14.
26. S. A. S. Wecinski. "*Solid Phase Microextraction, A practical Guide*", Marcel Dekker Inc., **1999**, New York, USA.
27. J. Pawliszyn, "Solid Phase Microextraction: Theory and Practice", Wiley, New York, USA, **1997**.
28. L. Anelli, F. Munari, A. Trisciani and J. R. Castanho. *Determination of Water Contaminants at Trace Levels Using Large-volume Sampling Techniques: Comparison of LVPTVI, LVOCI, and SPME*. Environmental Application Note, **May 2001**. http://www.iscpubs.com/articles/aln/n0105ane.pdf.
29. Y. Y. Shu, S. S. Wang, M. Tardif and Y. Huang. *J. Chromatogr. A*, **2003**, 1008, 1-12.
30. E. Baltussen, P. Sandra, F. David and C. Cramers. *J. Microcol. Sep*. **1999**, 11(10), 737-747.
31. R. Doong, S. Chang and Y. Sun. *J.Chromatogr. A*, **2000**, 879, 177-188.
32. H. Prosen and L. Zupančič-Kralj. *Acta Chim. Slov*. **1998**, 45(1), 1-17.
33. J. Beltran, F.J. López and F. Hernández. *J. Chromatogr. A*, **2000**, 885, 389-404.
34. G. Prokůpková, K. Holadová, J. Poustka and J. Hajšlová. *Anal. Chim. Acta*., **2002**, 457(2), 211-223.
35. M. Ábalos, J. M. Bayona and J. Pawliszyn. *J. Chromatogr. A*, **2000**, 873 (1), 107-115.
36. P. Braun, M. Moeder, P. Popp, P. Kuschk and, W. Engewald. *J. Chromatogr. A*, **2003**, 988, 41-51.
37. P. Okeyo, S. M. Rentz, and N. H. Snow. *J. High. Resol. Chromatogr*. **1997**, 20, 171-173.

38. P. Popp, C. Bauer, P. Keil and L. Wennrich. *J. Sep. Sci.* **2003**, 26, 961-967.

39. R. Doong and P. Liao. *J. Chromatogr. A*, **2001**, 981, 177-188.

40. F.J. López, E. Pitarch, S. Egea, J. Beltran and F. Hernández. *Anal. Chim. Acta.*, **2001**, 433(2), 217-226.

41. N. Fidalgo-Used, G. Centineo, E. Blanco-González and A. Sanz-Medel. *J. Chromatogr. A*, **2003**, 1017, 35-44.

42. A. Peñalver, V. García, E. Pocurull, F. Borull, and R.M. Marcé. *J. Chromatogr. A*, **2003**, 1007, 1-9.

43. F. David, A. Tredoux, E. Baltussen and P. Sandra. *Poster Presentation 23rd International Symposium on Capillary Chromatography-Riva del Garda*, Italy, **2000**, IOPMS.

44. C. Blasco, G. Font and Y. Picó. *J. Chromatogr. A*, **2002**, 970, 201-212.

45. S. Nakamura, N. Nakamura and S. Ito. *J. Sep. Sci.* **2001**, 24, 674-677.

46. B. Kolahgar, A. Hoffmann and A. C. Heiden. *J. Chromatogr. A*, **2002**, 963, 225-230.

47. P. Popp, C. Bauer, P. Keil and L. Wennrich. *J. Sep. Sci.* **2003**, 26, 961-967.

48. N. Ochiai, K. Sasamoto, S. Daishima, A. C. Heiden and A. Hoffmann. *J. Chromatogr. A*, **2003**, 986, 101-110.

49. T. Benijts, J. Vercammen, R. Dams, H. P. Tuan, W. Lambert and P. Sandra. *J. Chromatogr. B*, **2001**, 755, 137-142.

50. J. C. R. Demyttenare, J. I. S. Martínez, R. Verhé, P. Sandra and N. De Kimpe. *J. Chromatogr. A*, **2003**, 985, 221-232.

Chapter III

Instrumental aspects

Pollutants are generally present in trace and ultra-trace levels in nature ranging from micrograms per liter (ppb) to nanograms per liter (ppt). Hence, the most sensitive analytical instrumentation is required in environmental analysis and often measurements are done close to the detection limits of the systems. Capillary gas chromatography (CGC) is the most commonly used technique for routine analysis of environmental samples [1]. A brief overview of the principles of CGC is given below because it is the main technique employed in this work.

3.1. Capillary gas chromatography (CGC)

The chromatographic principle to separate compounds was first used by the Russian botanist M. S. Tswett in 1903 for the separation of plant pigments [2]. He used what would today be called liquid chromatography. This work was largely unnoticed for several decades before liquid chromatography appeared again in the 1940's. The gas chromatographic variant was developed a decade later by Archer, Martin and James (in 1952) [3,8]. Since then gas chromatography (GC) has developed very rapidly and it is today used in many different fields.

In general chromatography is defined as a technique in which the components to be separated are distributed between two non-miscible phases. One phase is stationary while the other is mobile and percolates through the latter in a definite direction. The chromatographic process takes place due to a continuous distribution process of the analytes of interest during the movement of the mobile phase through the stationary phase. The separation of a mixture of compounds is a result of differences in distribution constants among the individual sample components. The distribution constant (K) is defined mathematically by the Nernst distribution coefficient as follows [3 - 5]:

$$K = \frac{C_S}{C_M} \tag{3.1}$$

where C_S and C_M are the concentration of analytes in the stationary and mobile phase, respectively. In capillary gas chromatography (CGC), the mobile phase is an inert gas like He or H_2 and the stationary phase is generally a polymer with liquid-type properties immobilized on the capillary wall. The basic components of a CGC system comprise: a carrier gas supply, an injector, a column, an oven and a detector (Fig 3.1.).

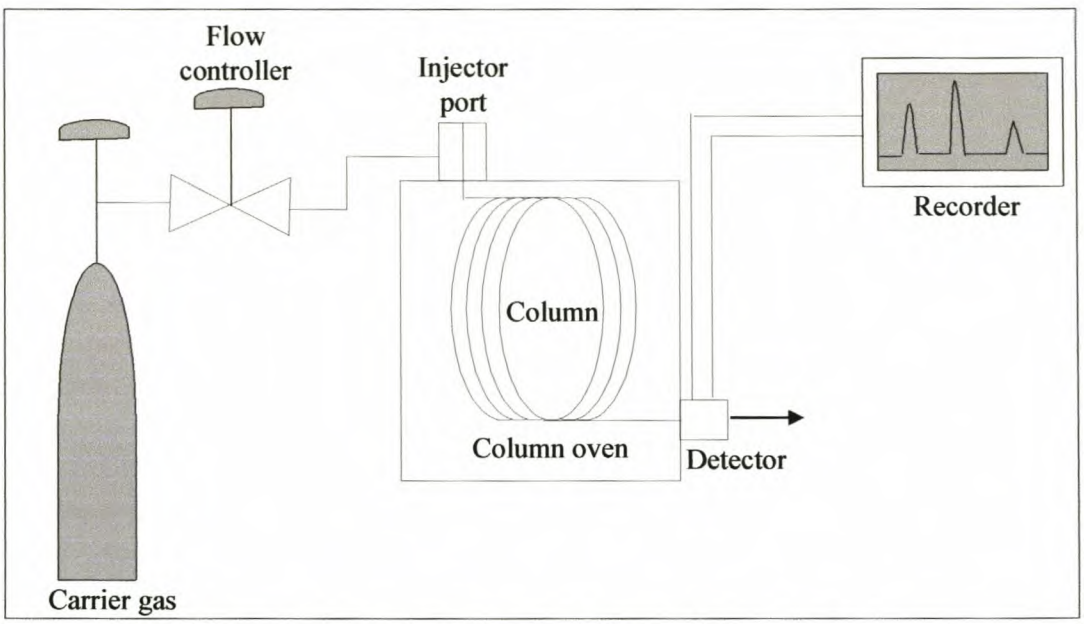


Fig 3.1. Schematic representation of a capillary gas chromatographic (CGC) system.

3.1.1. Carrier gas supply

The mobile phase (carrier gas) that carries the solutes through the system is mostly helium, hydrogen or sometimes nitrogen. Since the flow rate of the carrier gas affects the efficiency it must be carefully regulated. In older CGC systems the flow rate is controlled by a mechanical flow controller applying a constant pressure during the analysis. However, since the viscosity of a gas is a function of the oven temperature, this results in a decrease in linear velocity away from the optimum value. Additionally, with a mechanical flow controller it is difficult to reproduce exactly the same pressure. Therefore systems containing electronic pressure control are becoming increasingly popular. They allow the exact reproducibility of the column head pressure and they offer the possibility to work in the constant flow mode, whereby

one can work constantly at the optimal flow rate by electronically adapting the pressure [3,4,6].

3.1.2. The injector

The injector of the GC is a means of introducing the sample in the system while it vaporizes and mixes the sample with the carrier gas before entering the head of the column without causing any sample discrimination or degradation. Injectors can be universal or selective. Universal inlets such as split/splitless, cool on-column and programmable temperature vaporization (PTV) introduce the entire sample into the column whereas in selective injection only a fraction of the sample enters the column [2 - 4]. Injection can be performed either manually or automatically.

3.1.3. The oven

The oven has enough volume to hold the column easily. Typical GC ovens can be quickly and precisely heated to the preferred temperature varying from –100 °C to 450 °C at a rate of 0.1 °C/min to 50 °C/min. The atmosphere inside the oven has a very small inertia and is continuously agitated using forced ventilation. Very low temperatures can be obtained by using liquid N_2 or CO_2 through a cryogenic valve [3,4,6].

3.1.4. The column

Several types of column design are used in GC. These include: packed columns, capillary columns and support coated open tubular columns (SCOT). In packed and SCOT columns the stationary phase is deposited onto a porous support while in capillary columns the stationary phase is bound to the inner surface of the column. Today the capillary column is the most often used due to its superior separation efficiencies and is therefore the most suitable for environmental analysis. Fused silica open tubular columns with different film thickness of methyl silicone, methyl 5% phenyl silicone and methyl 50% phenyl silicone and many other stationary phases are commercially available [2 - 4].

3.1.5. The detector

The detector as one of the main parts of a GC 'senses' the compounds when they elute from the column. Detectors can be grouped into four types: ionization, bulk physical property, optical and electrical detectors according to the physical basis employed as the detection mechanism [3]. Detectors can also be broadly classified based on their response or selectivity: universal detectors, which are sensitive almost to all compounds in the mobile phase and selective or specific detectors, which are sensitive only to certain compounds i.e. the detector responses to a certain compounds. The most common universal detectors are: flame ionization detector (FID), thermal conductivity detector (TCD), and atomic emission detector (AED). Selective detectors include the nitrogen phosphorous detector (NPD), which is sensitive only to compounds containing nitrogen or phosphorous, the electron capture detector (ECD), which is sensitive only to electron capturing (example: halogenated) compounds and the flame photometric detector (FPD) that detects sulfur-containing compounds. The mass selective detector (MSD) can be universal when used in the full scan mode or selective when selected ion monitoring (SIM) is applied [2-4,7].

3.2. Instrumentation used in this work

The instrumentation used in this work comprises a thermal desorption-programmable temperature vaporization-gas chromatography-mass spectrometer (TD-PTV-GC-MS) and a gas chromatography-electron capture detector (GC-ECD). The components of these systems are discussed in detail below.

3.2.1. Split/splitless injector

A delicate operation in GC is sample introduction. Direct introduction of a large volume or of a concentrated sample can saturate the column, which can lead to a decrease in column efficiency and/or the production of distorted peaks. The oldest and most used injector in CGC is the split/splitless injector. A schematic drawing is shown in Fig 3.2. The injector was initially developed to introduce very small amounts of material (ng's) on the capillary column. This was done by splitting the flow of vaporized analyte molecules into a main flow that is discarded through a split valve

and a much smaller flow that is introduced on the column. In this way peak broadening due to overloading can be avoided [3,4].

Subsequently the splitless mode was discovered (by accident) whereby the split valve is closed for a few minutes during injection. This will easily lead to peak broadening due to column overloading and the long time required to transfer all the material to the column. However, these problems could be avoided by applying focusing mechanisms (mainly based on re-condensation effects) and by opening the split valve after a specific time.

The splitless injection mode is particularly suited for the analysis of trace compounds where it is advantageous to introduce all the material to the column. Hence, this mode is the one routinely used in this study.

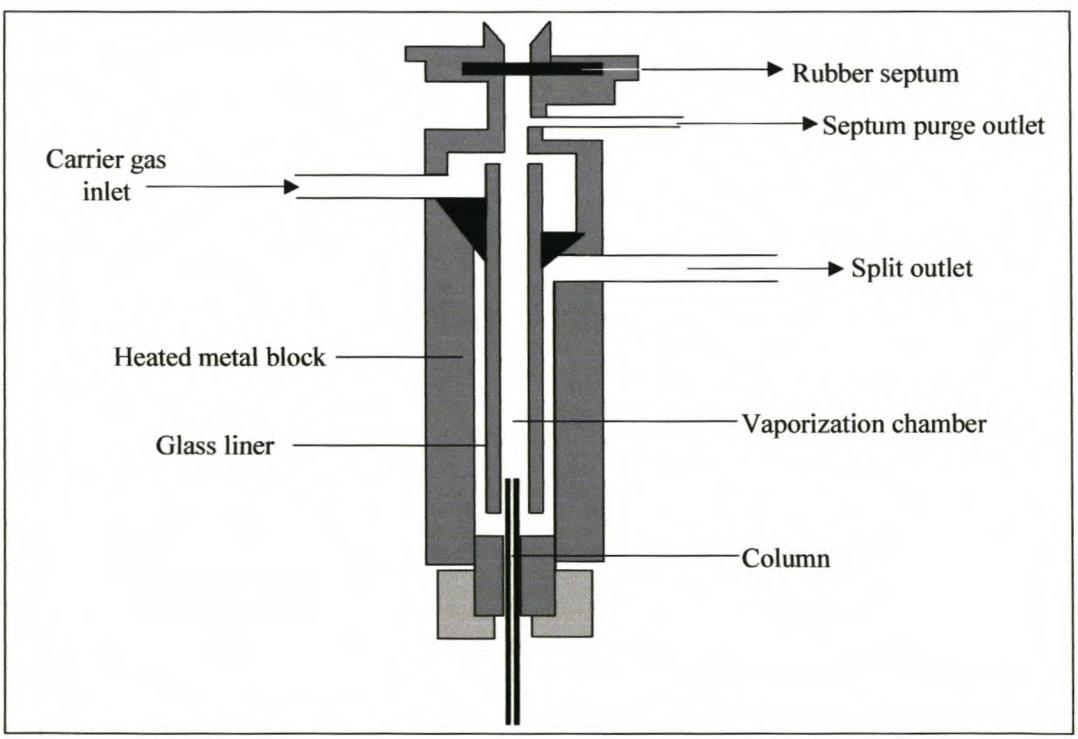


Fig 3.2. Schematic representation of the split/splitless injector.

3.2.2. Thermal desorption unit (TDU) and programmable temperature vaporization (PTV) injector

Due to the drawbacks of the classical split/splitless injector such as sample discrimination and/or alteration, a programmable temperature vaporization (PTV) injector has been developed [8,9]. This injector was found to show advantages by reducing the analyte discrimination during the injection step, by showing better recovery of thermo-labile compounds and by less pronounced adverse effects of non-volatile compounds present in the sample during the injection process [8]. It mainly differs from the classical split/splitless injector in the temperature control.

PTV can be used for both split and splitless injections. As mentioned, in splitless injection the sample is introduced at a temperature below or close to the boiling point of the solvent. The split exit is closed during sample evaporation and the solvent vapor enters the analytical column [8]. With a PTV the temperature can be heated or cooled rapidly by a sophisticated electronic control system using cold air, CO_2, or liquid nitrogen. The various parameters that can be adjusted are: initial temperature of the liner, temperature gradient, final temperature, carrier gas flow rate, and the use of either the split or splitless mode. Heating of the PTV can be performed by direct or indirect resistive heating. In this study this way of injecting was performed on a specially designed cooling injection system (CIS-4), which is meant for cryofocusing and fast injection of analytes desorbed in the thermal desorption system (TDS) into the capillary column [10].

The thermal desorption system (TDS) has been designed by Gerstel (GmbH, Germany) (Fig 3.3). It mainly consists of a removable desorption tube through which a carrier gas flows at a constant rate and a heating element for rapid heating of the chamber. The sorbents (e.g. PDMS) or adsorbents (e.g. Tenax) are placed in the tube. Sampling of gaseous or liquid samples can be done by pumping or sucking the sample (off-line) through the packed bed. For the thermal extraction of stir bars, they can be placed directly in the desorption glass tube. After desorption, the compounds are transferred to the PTV injector through a fused silica transfer column, which is kept at high temperature (>300°C) to prevent condensation of high molecular weight

compounds [6]. The solutes are then focused in the PTV by selecting an appropriate low temperature (<-100°C).

Depending on the nature of the analytes, or (ad)sorbents, the desorption conditions (initial temperature, temperature gradient, desorption temperature and carrier gas flow rate) can be adjusted to ensure complete desorption and transfer without sample or (ad)sorbent decomposition. Desorption can be carried out either in the split or in the splitless mode [6]. A schematic drawing of a TDS coupled to a PTV is shown in Fig 3.3. [11].

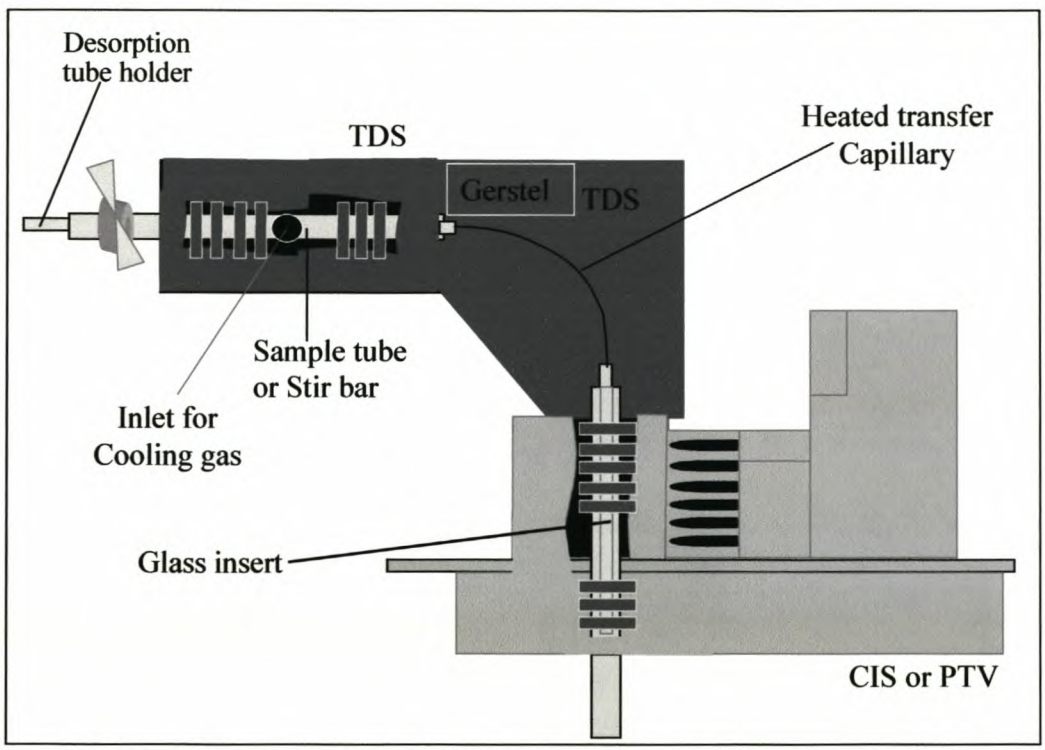


Fig 3.3. Thermal desorption system (TDS) coupled to a PTV injector.

3.2.3. The mass selective detector

A mass spectrometric detector or mass selective detector (MSD) is increasingly used routinely for environmental analysis in hyphenation to CGC. The MSD gives a histogram of the relative abundance of ions having different mass-to-charge (m/z) ratios generated from the sample molecules. In the MSD the process of ionization, the separation of the ions in a vacuum according to their m/z ratio, and the ion detection

are complex processes. The MSD is very helpful for identifying analytes because, in addition to the retention times where peaks of each compound are displayed in the chromatogram, it provides structural information of each compound in the form of a mass spectrum which can be compared to spectra listed in a computer stored library [3,6].

Mass spectrometers consist of three main parts: the ionization chamber, the mass analyzer and the detector. In the ionization chamber, the eluting analytes are ionized and fragmented into positive ions. Ionization of organic compounds can be done in many ways but the most commonly used with GC are: electron impact ionization and chemical ionization [2,3]. Only the former will be detailed.

The ionization chamber in electron impact ionization consists of a heated, evacuated chamber in which a beam of electrons with a narrow energy distribution is generated from a heated filament. Electrons with energies in the range between 5 to 100 eV can be used but 70 eV is standard practice. As most organic molecules have ionization potentials less than 20 eV, the energy transferred between the electrons and the neutral molecule is sufficient to create both ionization and fragmentation. Ionization takes place at a temperature sufficient to maintain the sample in the vapor phase at a pressure below 10^{-5} Torr, which is sufficient to ensure that the average mean free path of the ion is large enough for it to escape the source without undergoing a significant number of ion-molecule collisions. An electric field is used to accelerate the positive ions into the mass analyzer [3,6].

The most often used mass analyzer is the quadrupole (figure 3.4.), which separates ions according to their m/z ratio. It consists of four parallel metal rods in a square array such that the inside radius of the array is equal to the smallest radius of curvature of the metal rod. Diagonally opposite rods are attached electrically to radio frequency and direct current (dc) voltages. For a specific radio frequency/dc voltage ratio only ions of specific m/z value are transmitted and reach the detector. An electron multiplier is commonly used for the detection of the ions (Fig 3.4.) [3,6].

MS can be operated in two modes: the full scan mode (SCAN) and the selected ion-monitoring mode (SIM). In the full scan mode, as a result of changing the potential on

the rods continuously, fragment ions are separated in order of increasing masses. The continuous changing of the voltage (from low to high) while keeping the electric field constant is called a scan. The rate of scanning is in the order of 1-8 scans per second. The scan mode is used for the identification of unknown compounds in the environmental samples. On the other hand the SIM mode is used for specific ions only and gives better sensitivity than scan mode. In SIM mode the voltage does not vary except for the pre-selected values to permit only some ions with a specific m/z ratio to pass through the quadrupole thereby giving better sensitivity [6]. A schematic representation of a quadrupole mass spectrometer is given in Fig 3.4. below [12].

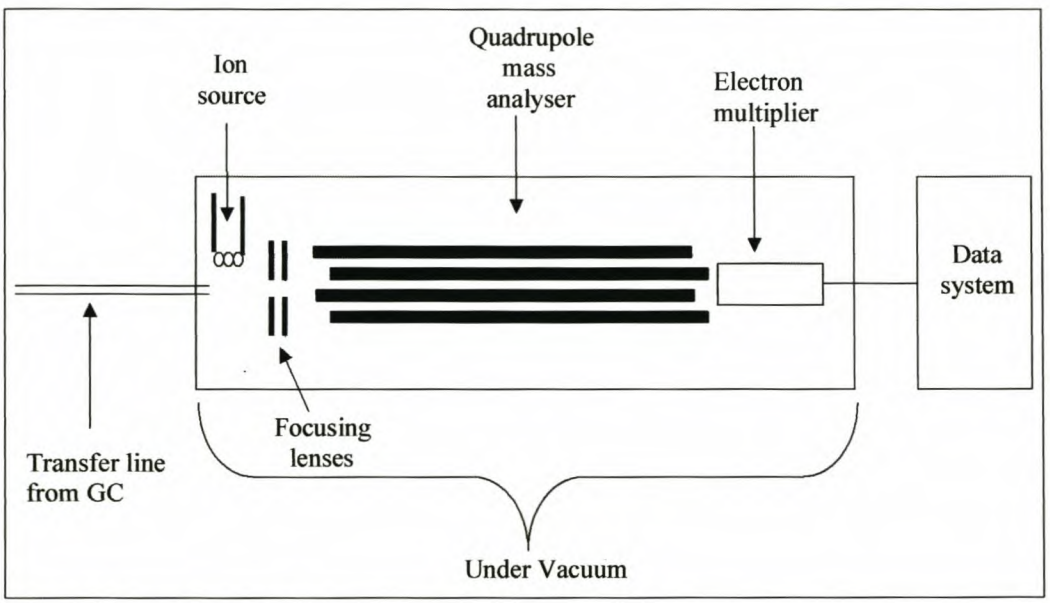


Fig. 3.4. Schematic of an MS system

3.2.4. The electron capture detector (ECD)

The ECD is the most widely used detector for the determination of electron capturing compounds like OCPs, polychlorobiphenyls (PCBs), dioxins and furans, trihalomethanes, etc. [2,3].

The ECD uses a low intensity radioactive source of beta particles (^{63}Ni), which collide with the detector make-up gas (nitrogen or argon/methane) to create many low energy (thermal) electrons. These low energy electrons are collected at the detector anode

and produce a small background or reference current. As electron-capturing analytes (halogenated compounds) elute from the chromatographic column, the thermal electrons are captured by these analytes, leading to a decrease in the current. In the state-of-the-art ECD's, the voltage across the cell electrodes can be pulsed to collect the remaining free electrons. The pulse rate changes to maintain a constant cell current; the change in pulse frequency is proportional to the analyte concentration [2,3,13]. The cross-section of an ECD is given in Fig 3.5.

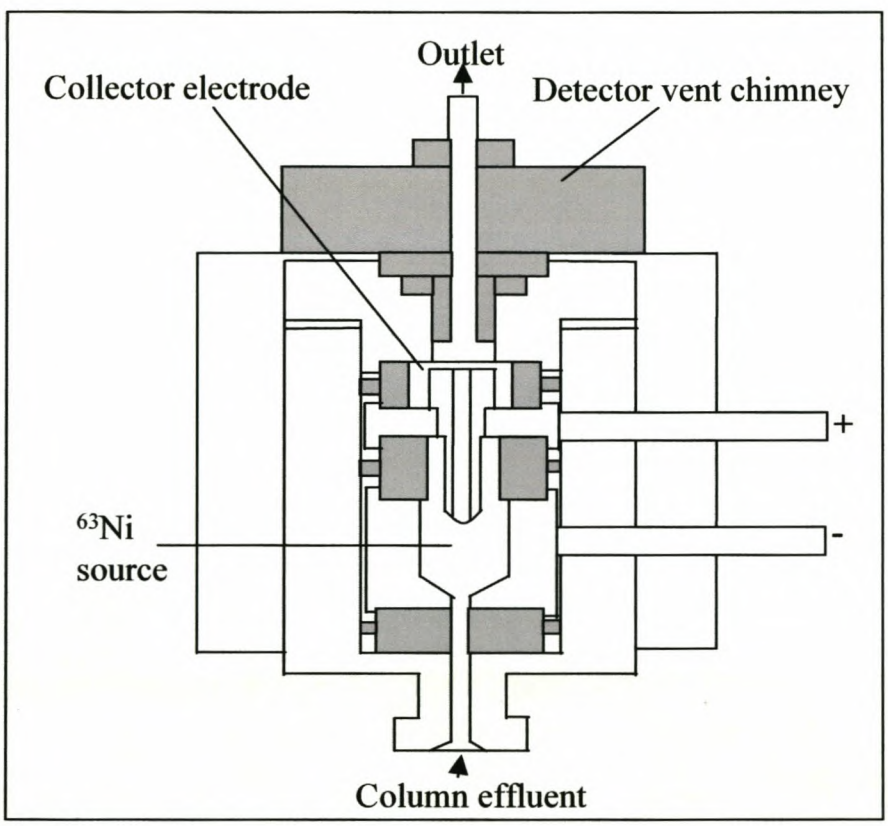


Fig 3.5. Schematic representation of an electron capture detector (ECD).

References

1. V. G. Berezkin, *chem. Revs.* **1989**, 89, 277.
2. P. Sandra, R. Soniassy and C. Schlett, *Water Analysis*, University of Stellenbosch, South Africa, **1998**.
3. C. F. Poole and S. K. Poole. *Chromatography Today*, Elsevier, **1999**, Amsterdam, The Netherlands.
4. F. Rouessac and A. Rouessac. *Chemical Analysis, Modern Instrumentation and Techniques*, **2000**, 5th edition, Wiley, New York, USA.
4. P. Molander. *The Influence of Temperature in Packed Capillary Liquid-Chromatography - Development and Applications*, PhD Dissertation, University of Oslo, Norway, **2000**.
6. A. G. J. Tredoux, P. J. F. Sandra and H. H. Lauer. *Stir Bar Sorptive Extraction. A Novel Technique To Monitor Micro-pollutants in Aqueous Samples*. MSc. Thesis University of Stellenbosch, South Africa, **2000**.
7. P. Sandra and F. Lynen. *Introduction to Separation Science*. Syllabus 3rd year BSc. Analytical Chemistry, University of Stellenbosch, South Africa, **2003**.
8. M. Godula, J. Hajšlová, K. Maštouska and J. Křivánková. *J. Sep. Sci.* **2001**, 24, 355-366.
9. W. Vogt, K. Jacob, A. Ohnesorge and H. W. Obwexer. *J. Chromatogr.* 1979, 186, 197-205.
10. F. David, B. Tienpont, P. Sandra and R. E. Majors. *LC-GC North America*, **2003**, 21, 1-7.
11. A. Hoffmann, J. Kames and J. Steinhanses. Gerstel *AppNote 3/1996*, **1996**.
12. R. L. Grob. *Modern Practice of Gas Chromatography*, **1995**, 3rd edition, Wiley, New York, USA.
13. M. E. Walsh. *Talanta*. **2003**, 54, 427-438.

Chapter IV

Development of three analytical methods for the analysis of OCPs in water samples

The analysis of OCPs in water samples requires an extraction step. This in order to remove the aqueous matrix, which is not compatible with GC, and to pre-concentrate the analytes to increase the sensitivity of the method as is generally necessary in environmental analysis. In this chapter three different sample preparation methods and techniques for the analysis of OCPs by GC-ECD and GC-MS were evaluated. The performance of the different methods is compared in Chapter V. The finalized instrumental conditions are given at the beginning of each section.

4.1. Reagents and materials

A standard mixture containing 2000 µg/ml of the 17 OCPs shown in Table 1.1, 1.2 and 1.3 (with the exception of Endrin ketone) dissolved in toluene:hexane (50:50, v/v), was used for calibration purposes (Supelco, Bellefonte, PA, USA). These standards were stored at 4°C and used for the preparation of a working standard solution. Pentachloronitrobenzene 99% (as an internal standard, IS) was purchased from Aldrich (Steinheim, Germany). Hexane (pestanal purity grade ≥ 99%) for residue analysis was purchased from Fluka (Zwijndrecht, Netherlands). Acetone, Methanol, Toluene and Dichlorodimethylsilane (DMDS) were purchased from Aldrich (Steinheim, Germany). Distilled water was provided from the University of Stellenbosch.

Special glass tubes (vials) for µLLE were manufactured in the Chemistry Department, University of Stellenbosch. The SPME holders for manual use and the 100 µm PDMS fibers, the 15 ml glass vials and the 45 ml glass vials, were obtained from Supelco (Bellefonte, PA, USA). Stir bars with 0.5 mm film thickness (Twister®) were purchased from Gerstel (Gerstel, Müllheim a/d Ruhr, Germany).

Environmental samples (water, soil and sediment) were collected from Eritrea and stored at 4°C prior to analysis. Moreover, water and soil samples for the testing of the developed methods were collected from Stellenbosch farmland.

All glassware used in this work was washed with a detergent solution, distilled water and acetone. To minimize the sorption effect of OCPs on the glassware, all vials and glass tubes were soaked overnight in a chromic acid solution followed by a treatment in a 10 % (v/v) mixture of dichlorodimethylsilane (DMDS) in toluene for 30 min to block all active sites on the glassware. Finally, the glassware was rinsed with methanol and oven dried at 280°C.

4.2. Initial experiments by direct injection

The first step in the method development was to evaluate the performance of the instrument in use by direct injection of pesticide standard solutions. This in order to assess the linearity, repeatability and sensitivity of the system and to optimize the separation conditions.

4.2.1. Finalized instrumental conditions

After optimization of the different parameters such as obtaining fully resolved peaks, low base line, and good sensitivity, the following optimized instrumental conditions were used.

A Fisons instrument 8000 series Gas Chromatograph equipped with a split/splitless injection port and an electron capture detector (ECD 400) was used for the analyses of the OCPs. The head pressure of the carrier gas (helium) was set at 180 kPa corresponding to a flow rate of 3.0 ml/min (63.5 cm/sec) at 70 °C. Injection was operated in the splitless mode. The splitless time was set at 1 min. The injector was maintained at 250 °C. A 30 m ZB-5 capillary column (0.250 mm I.D. 0.25 μm film thickness, Phenomenex, U.S.A) was used for separating the pesticides. The column was held at 70 °C for 2 min, and then the

temperature was increased to 150 °C at a rate of 25 °C/min, at 3 °C/min to 200 °C, followed by a rate of 8 °C/min up to 280 °C where it was held for 10 min.
The ECD was operated at 300°C in the constant current mode with a pulse voltage of 5V and a reference current of 0.5 nA. The pulse width was set at 1 μs and 0.1 μs for N_2 and Ar/CH_4 make-up gas, respectively. The make-up gas pressure was set at 150 kPa.

4.2.2. Initial experiments

A chromatogram obtained for standards of the 17 OCPs analyzed after optimization of the temperature program and the column head pressure is shown below for a 1 μl injection containing 10 ppb of each of the pesticides in hexane (Fig 4.1.).

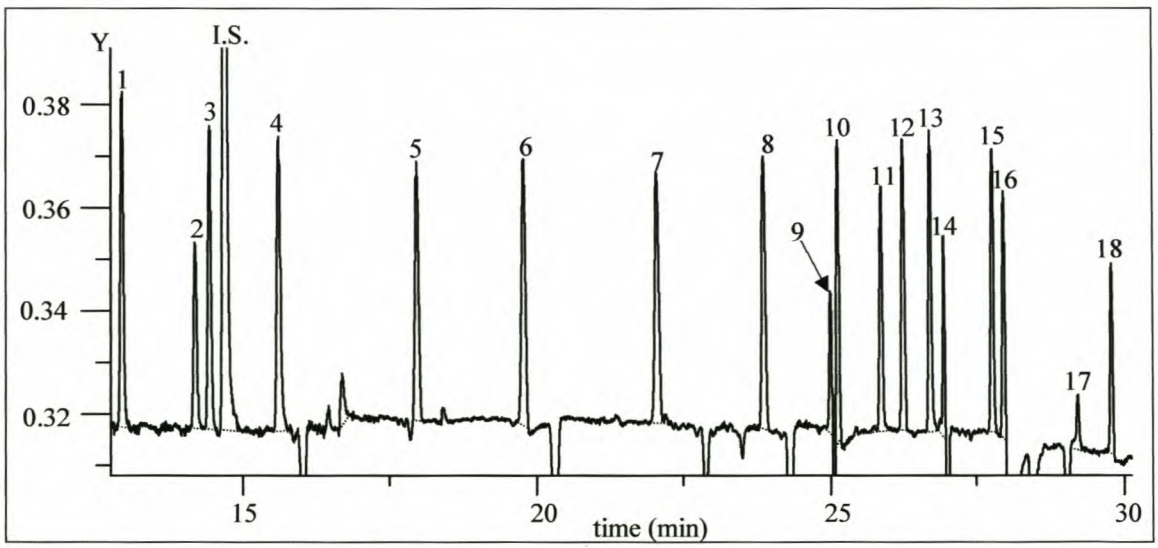


Fig 4.1. GC-ECD chromatogram of 10 ppb of 17-OCPs obtained under N_2 make-up gas. 1. α-BHC, 2. β-BHC, 3. γ-BHC, 4. δ-BHC, 5. Heptachlor, 6. Aldrin, 7. Heptachlor epoxide, 8. Endosulfane I, 9. Dieldrin, 10. p,p'-DDE, 11. Endrin, 12. Endosulfane II, 13. p,p'-DDD, 14. Endrin aldehyde, 15. Endosulfan sulfate, 16. p,p'-DDT, 17. Endrin ketone, 18. Methoxychlor. Concentration I.S. (pentachloronitrobenzene): 100 ppb. **NB:** Endrin ketone is a degradation product of Endrin.

Although Endrin ketone was not present in the sample it inevitably appeared in the chromatograms due to the easy degradation of endrin into ketone isomer (in the standard mixture stored in the fridge and in the injection process) and this was, hence, hard to avoid.

Also notice in Fig 4.1. that, due to the noisy base line, the signal to noise (S/N) ratio is rather low. This limits the limit of detection (LOD) because the latter is generally defined as a S/N ratio of 3 and also the limit of quantification (LOQ), which is set at a S/N ratio of 10.

It was possible to detect OCPs below the 1 ppb level under these initial conditions. The linearity of the detector was investigated in the range between the 1 ppb and 500 ppb at six point calibration levels (1 ppb, 10 ppb, 50 ppb, 100 ppb, 250 ppb and 500 ppb). However, a relatively small linear range was observed. Since for most of the compounds 500 ppb was already out of the linear range, it is not shown in the calibration graph of Fig. 4.2. The r^2 values were found greater than 0.99 for most of the OCPs between 1 and 250 ppb. The repeatability of the injections (n = 4) was calculated in terms of percent relative standard deviation (% RSD) of the absolute peak areas and these ranged from 5.22% to 22.49%.

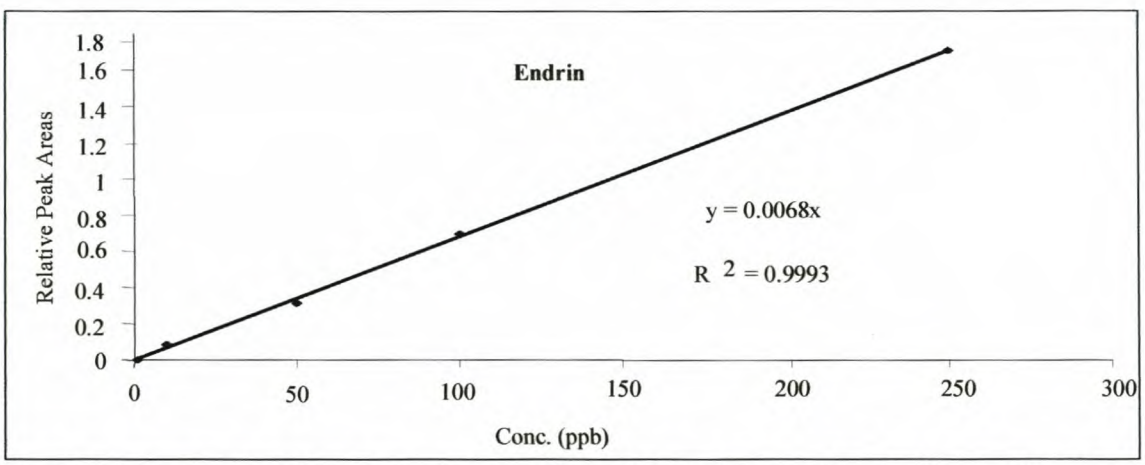


Fig 4.2. Calibration line of endrin by direct injection of 1 µl of 1 ppb, 10 ppb, 50 ppb, 100 ppb, and 250 ppb OCP standard solutions analyzed by CGC-ECD using N_2 as make-up gas (500 ppb is out of linear range).

Hence, it is clear from figure 4.1 and 4.2 that the sensitivity and linear range of the method are insufficient for the analysis of genuine environmental samples due to the very low amount of OCPs in the environment, which require a highly sensitive method and sample enrichment.

However, after optimization of the ECD conditions in terms of cleaning the collector electrode, varying the pulse voltage, reference current and ECD temperature, it was also observed that a mixture of 10% methane in argon generated a much lower base line and reduced negative base-line depth compared to the ultra pure N_2 originally used as make-up gas. This can be seen in Fig 4.3. for the analysis of the same pesticide samples.

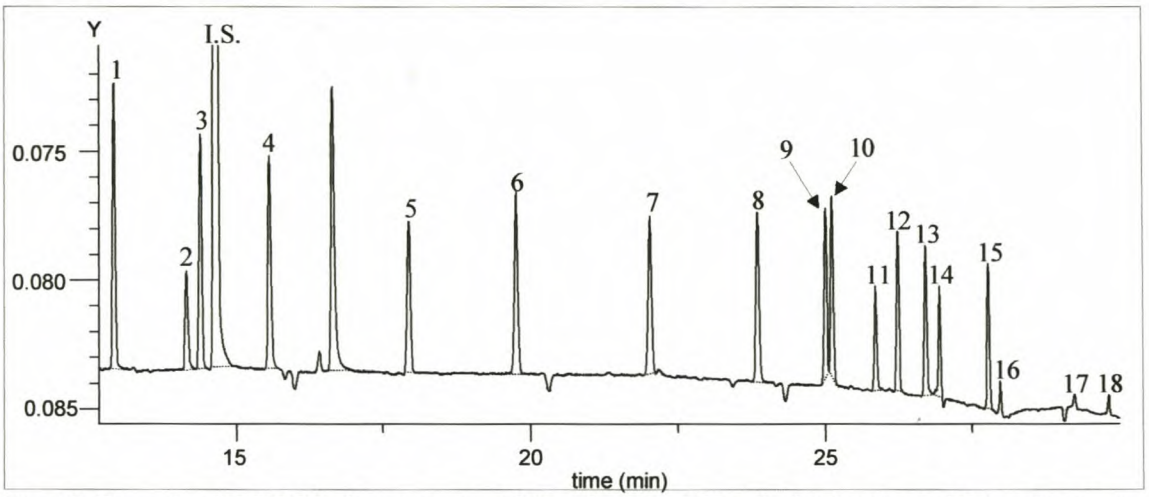


Fig 4.3. Example of GC-ECD chromatogram of 10 ppb of 17-OCPs obtained under Ar/CH_4 make-up gas. **NB:** Compound identities see Fig 4.1.

Under these conditions the linear range improved to a range of $1:10^3$ as can be seen in Fig 4.4 and the repeatability of the injections was ranging from 0.34% for aldrin to 7.57% for endrin aldehyde.

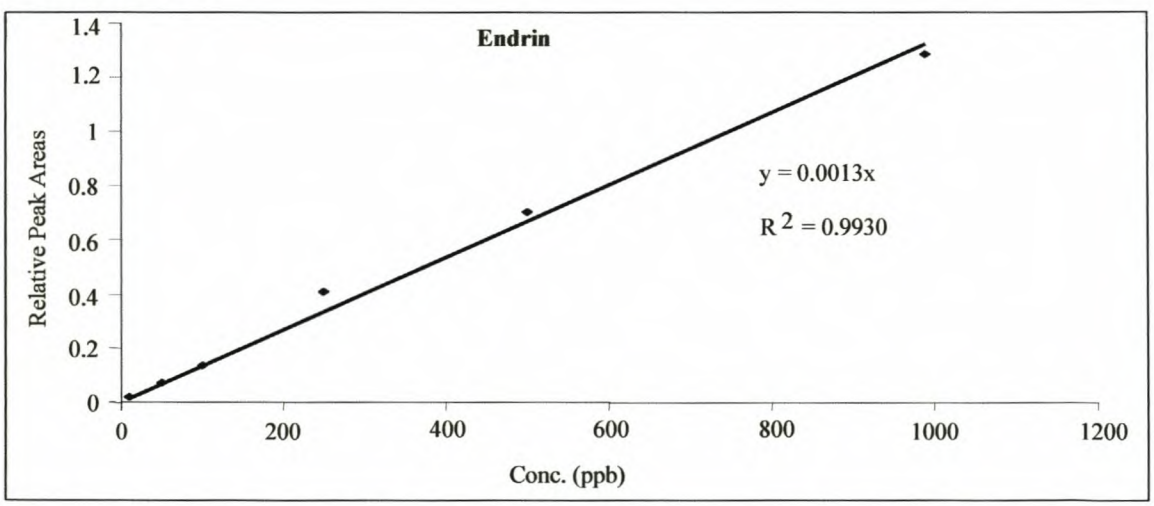


Fig 4.4. Calibration line of endrin by direct injection of 10 ppb, 50 ppb, 100 ppb, 250 ppb, 500 ppb and 1000 ppb OCP standards analysed by CGC-ECD using Ar/CH_4 as make-up gas.

These preliminary assays, hence, allowed the optimization of the separation and detection limits to allow maximum sensitivity. It is, however, clear that direct injection of pesticide mixtures is not yielding sufficient sensitivity for all 17 OCPs. Furthermore some kind of extraction technique is in any case required due to the fact that it is not possible to inject aqueous samples as such. For these reasons several extraction and pre-concentration techniques were investigated.

4.3. Development of the μLLE method

The first extraction technique investigated was micro liquid-liquid extraction (μLLE) because of its well-established character and ease of operation. For reasons outlined in Chapter II, the miniaturized version of LLE, micro liquid-liquid extraction (μLLE), was examined. The μLLE method was developed for the extraction of the 17-OCPs from spiked water using hexane in micro-liter amounts followed by CGC-ECD analysis.

4.3.1. Optimized μLLE procedure

Specific concentrations of standard pesticides and internal standard (I.S.) were placed in home-made glass tubes shown in Fig 4.5. The solvent (in this case hexane) was evaporated under N_2. Deionized water (5 ml) was added and sonicated in an ultrasonic bath for 20 min (so as to get good mixing of the pesticides in the deionized water). Then 200 μl of hexane was subsequently added, shaked mechanically for 2 min and centrifuged for 5 min. From the organic phase (the top layer), 100 μl was transferred to a 1.5 ml glass vial and 1 μl of the extract was manually injected on the GC-ECD system using a 10 μl syringe.

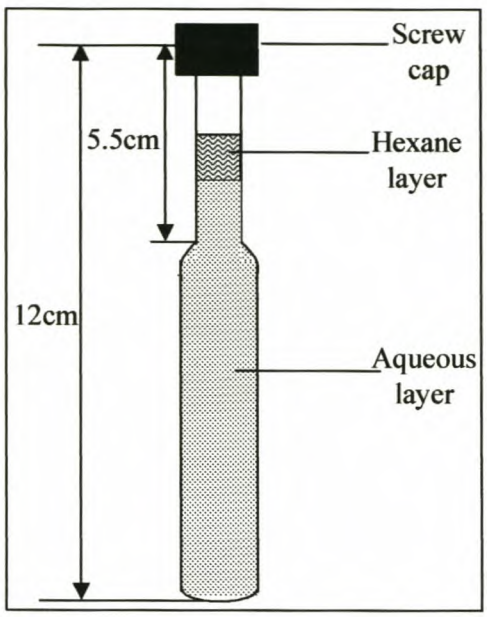


Fig 4.5. Schematic representation of the home-made glass tubes used for μLLE.

4.3.2. Finalized instrumental conditions

The instrumental conditions for μLLE-CGC-ECD have been outlined in section 4.2.1.

4.3.3. Development of the method

In the case of μLLE, assuming that 100% recovery is obtained and since the extraction was done with 200 μl of hexane from 5 ml (5000 μl) of deionized water, a concentration factor of 25 is expected to be achieved i.e. 5000 μl divided to 200 μl gives 25.

To check the linearity of the μLLE method five concentrations (0.1 ppb, 1 ppb, 5 ppb, 10 ppb and 20 ppb) of the pesticide calibration samples were prepared in 5 ml deionized water. When all are extracted in 200 μl of hexane, the concentration of the pesticides in the organic layer becomes 2.5 ppb, 25 ppb, 125 ppb, 250 ppb and 500 ppb, respectively. With N_2 as make-up gas in the ECD, the linear range was found to be small and by changing to the Ar/CH_4 mixture, the linearity showed much improvement with a good r^2-value and a range of $1:10^3$. This is shown for β-BHC in Fig 4.6. below.

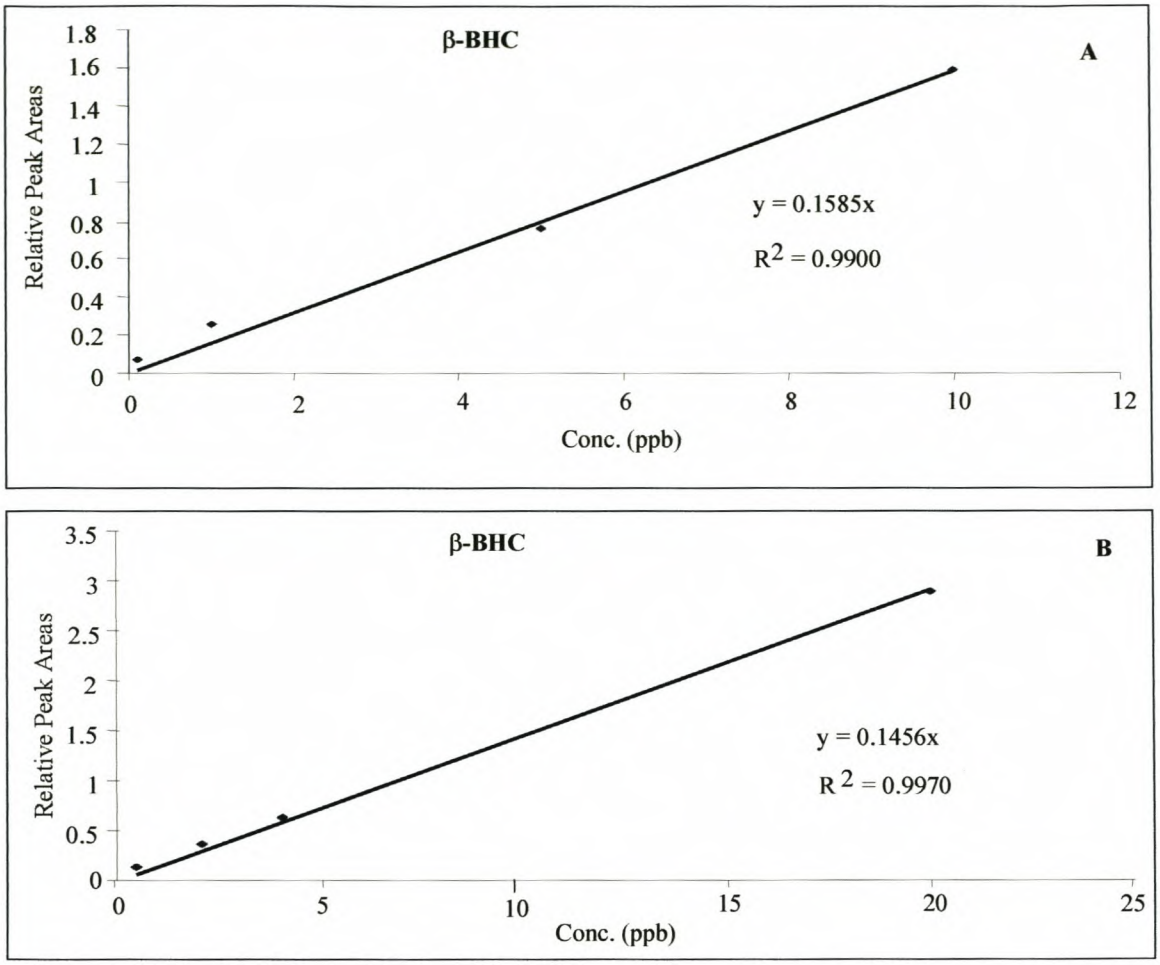


Fig 4.6. Calibration lines for β-BHC by μLLE-CGC-ECD method (A) using N_2 as make-up gas, (B) Ar/CH_4 as make-up gas.

The use of μLLE in combination with GC-ECD analysis allows lowering of the detection limits down to 100 ppt. This is the maximum allowable level for most of the analyzed OCPs as set by the European Union (EU) and American Environmental Protecting Agency (EPA) for drinking water. As can be seen in Fig 4.7. all the OCPs can be seen and identified clearly at this level even when using N_2 as make-up gas.

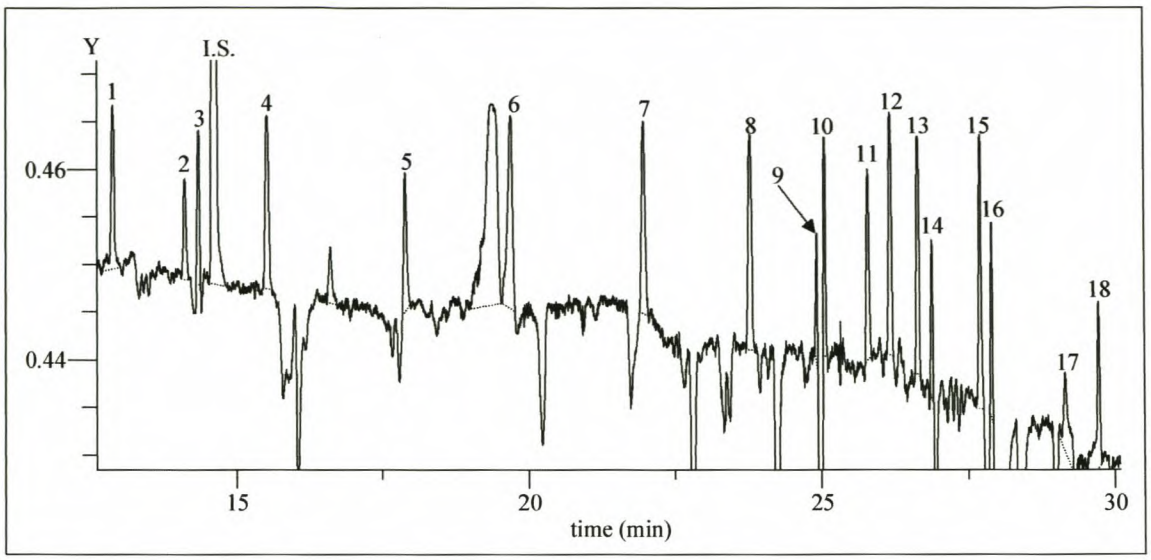


Fig 4.7. Chromatogram of 100 ppt standards using µLLE-CGC-ECD obtained under N_2 make-up gas. **NB:** Compound identities see Fig 4.1.

Again a better S/N ratio, reduced and less noisier base-line chromatogram was found when using Ar/CH_4 as make-up gas. This is clear from Fig 4.8. although a 400 ppt sample was investigated in this case.

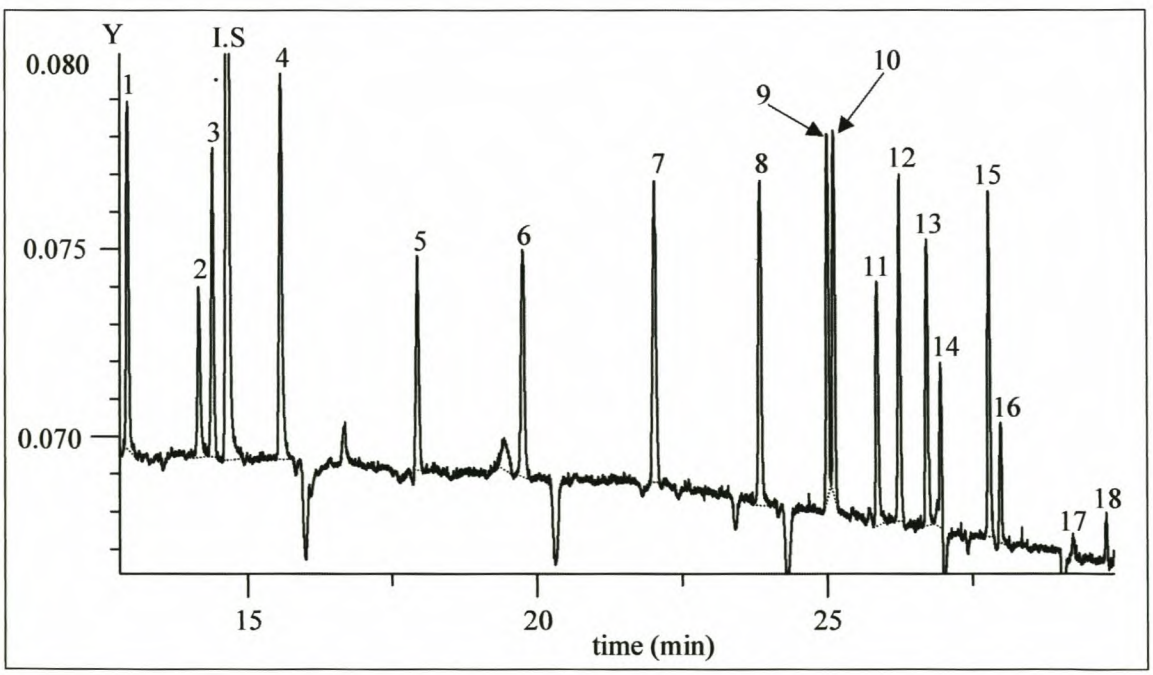


Fig 4.8. Chromatogram of 400 ppt standards using µLLE-CGC-ECD analyzed under Ar/CH_4 make-up gas. **NB:** Compound identities see Fig 4.1.

The LOD was found to be less than 100 ppt and, correspondingly, the LOQ less than 300 ppt, for all 17 OCPs. The repeatability (%RSD) of the injections for the μLLE was found ranging from 3% for γ-BHC to 19% for methoxychlor, which is comparable to values reported in the literature [1].

Upon application of this method to real environmental water samples it was observed that artifact signals were often appearing due to matrix effects. These could be removed by increasing the pH of the samples but this also resulted in the loss of some of the OCP peaks. The simplest solution was found by dilution with deionized water prior to extraction (1:2 v/v). In the example shown in Fig 4.9. it can be seen that three OCPs were detected. Quantification was done through external calibration.

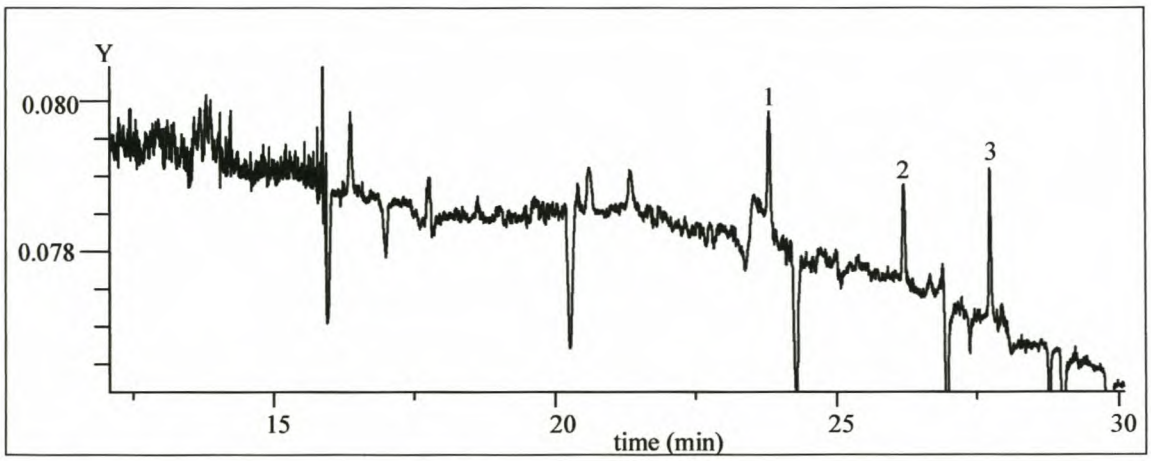


Fig 4.9. CGC-ECD chromatogram of μLLE extract of a real environmental water sample collected from Stellenbosch farmland. The pesticides detected were 1) Endosulfan I = 508 ppt, 2) Endosulfan II = 229 ppt, 3) Endosulfan Sulfate = 604 ppt, respectively.

Even though μLLE is a fast, simple and cheap technique that can detect down to the maximum tolerance of OCPs in drinking water as set by the European Union (100 ppt), the amount of the OCPs in the environment is generally very low. Therefore in order to quantify OCPs in environmental samples, more sensitive sample preparation methods that can provide a very low concentration in sub ppt level are required. Moreover μLLE still uses small amounts of organic solvent. Therefore solvent free sample preparation methods were investigated.

4.4. Development of the SPME method

Due to the above-mentioned shortcomings and because of the solventless aspects of sorption techniques, a solid phase microextraction (SPME) procedure was investigated for the determination of the 17 OCPs in environmental water samples. The extracted samples were analyzed by CGC-ECD. Parameters affecting the sorption of analyte into the polydimethylsiloxane (PDMS) fiber, and especially sampling time (30 min, 45 min, 1 h, and 2 h) were examined. All the analyses carried out at room temperature. The method was tested with real environmental water samples.

4.4.1. Optimized SPME procedure

SPME was performed by adding a specific concentration of the pesticides and the internal standard (pentachloronitrobenzene), both dissolved in hexane, into a 15 ml glass vial. The solvent was evaporated under N_2. To the vial 7 ml deionized water was added and sonicated for 20 min. A glass-lined magnetic bar was used as a stirrer to agitate the solution at 700 rpm. SPME was conducted by immersing the fiber into the aqueous phase (direct SPME) with stirring at room temperature for 1h. After extraction, the fiber was thermally desorbed for 5 min in the GC injector at 250 °C ensuring complete removal of the analytes. This was assured by re-inserting the SPME fiber in a next run resulting in a blank analysis. Blank analyses were done periodically during the course of this work to confirm the absence of contamination. The 100 μm PDMS coated fiber was conditioned in the GC injector for 30 min. at 250°C according to the instructions provided by the manufacturer.

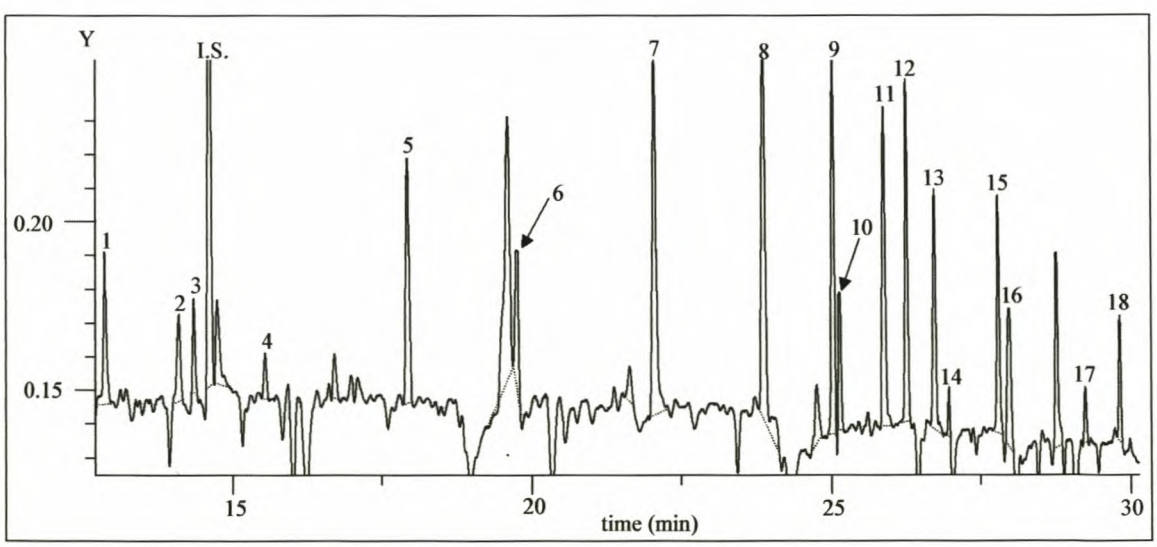
4.4.2. Finalized instrumental conditions

The instrumental conditions are identical to those used in the μLLE-CGC-ECD method and have been discussed in section 4.2.1.

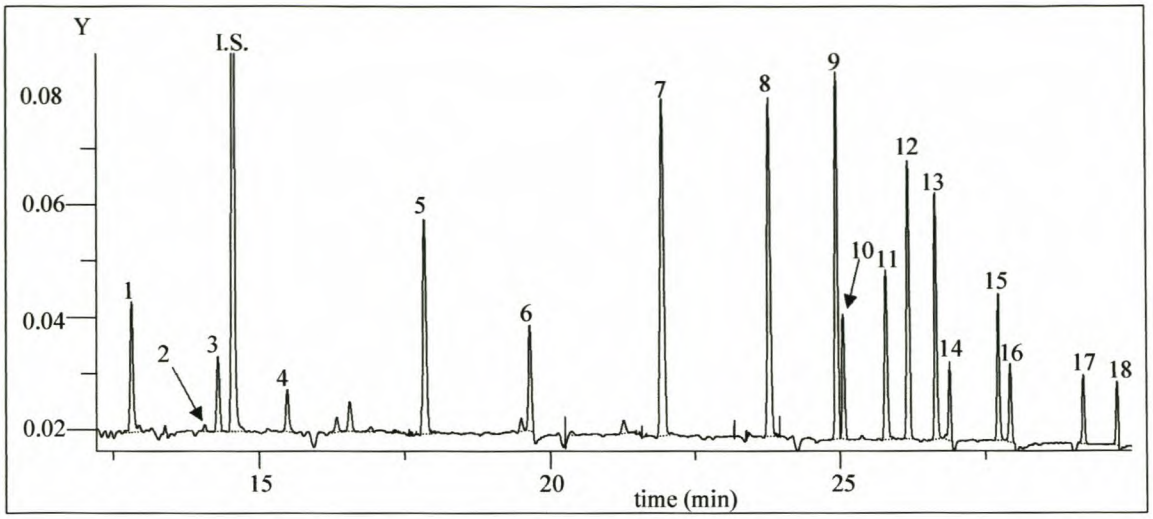
4.4.3. Development of the method

The efficiency of SPME also depends on the equilibration time. Longer equilibration time reflects slow kinetics of the SPME process. The longer the equilibration time the more high molecular mass compounds will be enriched on the PDMS fiber as they have lower diffusion coefficients. From the examined equilibration times (30 min, 45 min, 1 h, and 2 h), 1 h as reported [2], was selected as optimum because no additional recovery was observed when extraction time was increased beyond this time. The 30 min and 45 min samplings showed less intense peaks.

The SPME method allows sensitivities down to 1 ppt. However, few of the peaks such as peaks # 2, 6, 14, 17 and 18 (Appendix A, Fig. 2 and 3) disappear due to their lower affinity towards the fiber. Also with the SPME method using Ar/CH_4 as make-up gas better signals, lower base-line and reduced negative base-line depth were obtained compared to N_2 (Fig 4.10. (a) and (b)).



(a)

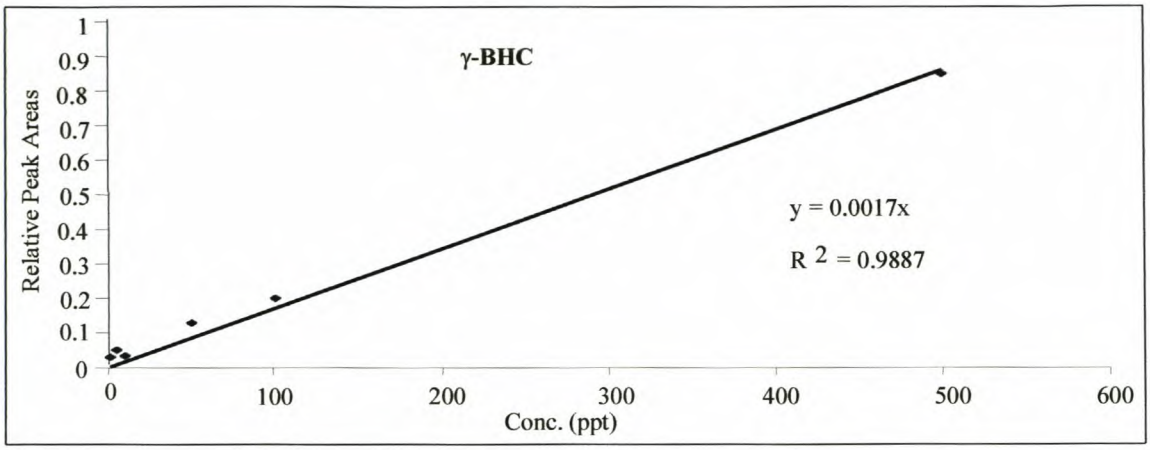


(b)

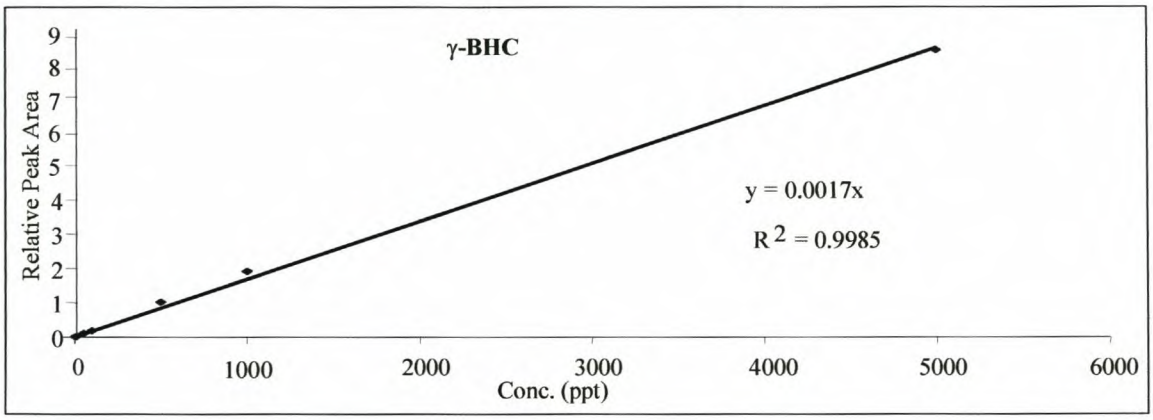
Fig 4.10. Chromatograms of SPME-CGC-ECD of 50 ppt OCPs standards using (a) N_2 as make-up gas, (b) Ar/CH_4 as make-up gas. **NB:** Compound identities see Fig 4.1.

The linearity of the SPME-GC-ECD method was evaluated by preparing nine different OCP calibrating solutions (1 ppt, 5 ppt, 10 ppt, 50 ppt, 100 ppt, 500 ppt, 1000 ppt, 5000 ppt and 10,000 ppt). The concentrations above 500 ppt analyzed by using N_2 as make-up gas were found to be out of the linear range limiting the range to 500. However with Ar/CH_4 as make-up gas the linearity showed a good range of 1:5000, a 10 fold improvement (Fig 4.11.).

The repeatability of the method was evaluated by doing series of five injections from the same concentrations and expressing the peak areas in terms of percent relative standard deviation (%RSD). Except p,p'-DDE, with %RSD of 23% and p,p'-DDT with %RSD of 38%, for the other pesticides the deviations varied between 5% and 12%, which values are similar to the ones reported in the literature [3].



(a)



(b)

Fig 4.11. Calibration lines of γ-BHC by SPME-GC-ECD method using (a) N_2 as make-up gas, (b) Ar/CH_4 as make-up gas.

The application of the SPME method to real water samples was attempted with water samples collected from Stellenbosch farmland. For some of the OCPs, it was observed that the recoveries on the PDMS fiber were strongly dependent on the pH [4]. This was especially observed for the endosulfan group. Moreover, large interferences from non-target analytes were observed. To minimize this problem it was attempted to decrease the pH by adding 0.1M H_2SO_4, to increase the pH of the samples by adding 0.1M NaOH and diluting the samples with deionized water prior to extraction. Dilution of the sample with deionized water prior to extraction was found to be the best way to minimize the matrix effects. In Fig 4.12. a comparison of an unmodified and diluted of the same water sample is shown.

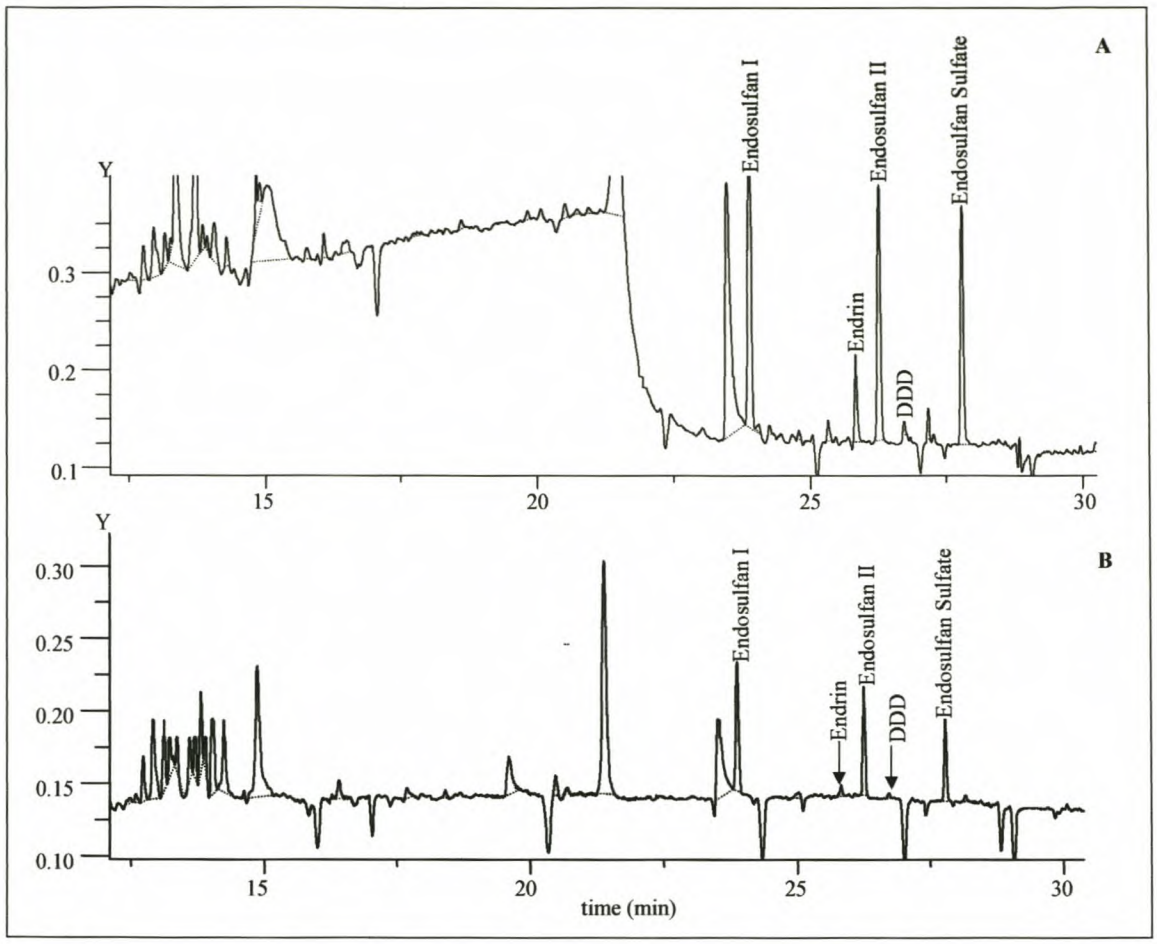


Fig 4.12. Chromatogram of a water sample collected from the Franschhoek area. (A) without modification, (B) after dilution with deionized water to 1:10. Analysis was done by SPME-CGC-ECD using N_2 as make-up gas.

It can be seen from Fig 4.12. that dilution gives qualitatively good results, by minimizing the matrix influence in general without affecting the OCPs as such. The identity of the compounds covered by the large area up to the retention time of 22.5 min in Fig 4.12A was investigated by analyzing the dirty water sample using SBSE-TDS-PTV-GC-MSD (see further). The results showed that the large peak is composed of a mixture of carboxylic acids, phenolic compounds, nonylphenols, straight chain alkenes and alkynes, apparently related to oil and detergent contamination in the water sample. A similar situation was observed for a water sample collected from the Stellenbosch area (see Fig 5. Appendix B).

It is clear from the above shown results that the combination of SPME sample preparation, GC separation and ECD detection provides an extremely sensitive technique for the OCPs under study. Problems with contamination, as experienced in the μLLE were almost non-existent in the SPME method. It is, however, clear that the lack of identification power of the ECD system other than based on retention time can sometimes cast some doubt on the identity of the observed peak, specially in the case of heavily contaminated matrices. Therefore mass spectrometric detection was investigated. However, because it is known that MS detection is less sensitive than ECD detection, another and more powerful sample preparation technique, stir bar sorptive extraction (SBSE), was combined to the system. In this way it was expected to hyphen the superior identification power of the MS detection without loss of sensitivity.

4.5. Development of SBSE method

SBSE is a recently developed technique for sample enrichment and has been evaluated for analysis of pesticides in aqueous samples (drinking water and beer [4] and in wine [5]). In this study it was investigated for OCPs extraction from natural water systems.

4.5.1. Optimized TDS-PTV-GC-MS conditions

The instrumental set-up is similar to the one described by Sandra et al. [6,7]. GC-MS was carried out with an Agilent 6890 GC coupled to a 5972 MS (Agilent Technologies, Palo Alto, CA, USA) equipped with a TDS-2 thermodesorption system (Gerstel GmbH, Mülheim a/d Ruhr, Germany). A CIS-4 PTV injector (Gerstel GmbH) was used for cryofocusing the analytes prior to transfer onto the analytical column. The CIS-4 was equipped with an empty baffled glass liner. Liquid nitrogen was used to cool the CIS-4 down to –100 °C during thermal desorption. SBSE desorption was started at 40 °C and the temperature was raised at 60 °C/min to 300 °C. This temperature was held for 5 min under a flow of 80 ml/min helium. The CIS injector temperature was ramped from –100 °C to 280 °C, at 12 °C/s and held there for 5 min. The heated transfer line was set at 325 °C. Helium was used as the carrier gas with a pressure of 1 bar (100 kPa) and a column

flow rate of 1.2 ml/min in the constant flow mode. The analysis in both the TDS-2 and CIS-4 was done in the splitless mode. A 30 m ZB-5 capillary column (0.250 mm I.D. 0.25 μm film thickness, Phenomenex, Torrance, CA, U.S.A) was used for separating the pesticides. The column was held at 70 °C for 2 min, increased to 150 °C at a rate of 25 °C/min and again ramped at 3 °C/min to 200 °C, followed by an increase in temperature to 280 °C at a rate of 8 °C/min, and kept there for 15 min. The MS transfer line temperature was kept at 280 °C. The MS was operated in the EI mode with a scan range of m/z 50 to 300 at 2.94 scans/s. In the SIM mode, three monitoring ions for each compound were selected. The ions were monitored with a dwell time of 100 ms per ion.

4.5.2. Finalized SBSE procedure

A known concentration of OCPs and internal standard prepared in deionized water was transferred to a 40 ml glass vial. The volume was made up to 25 ml by adding deionized water. The mixture was homogenized in an ultrasonic bath for 15 min. A preconditioned SBSE stir bar (Twister®, Gerstel, Müllheim a/d Ruhr, Germany) of 10 mm length coated with a 0.5 mm PDMS layer, was added and stirred for 1 h at 30 °C and 1250 rpm. After sampling the stir bar was removed with tweezers, placed on a tissue to dry and remove residual droplets and finally placed in the liner of the thermal desorption system for analysis. The stir bars were re-conditioned at 280 °C with a nitrogen stream flow and no carry-over was observed.

4.5.3. Development of the analytical method

Because of the high vacuum in the MS, the compounds elute at slightly earlier retention times (but with the same profile) in GC-MS compared to GC-ECD under the same operational conditions. This required some minor alteration in the helium flow rate in order to maintain the baseline separation of all peaks. Hence, the first task was to check the position and retention times of each compound by identifying them in the scan mode. The optimized separation with the identified compounds and, as example, the spectrum of p,p'-DDT is shown in Fig 4.13.

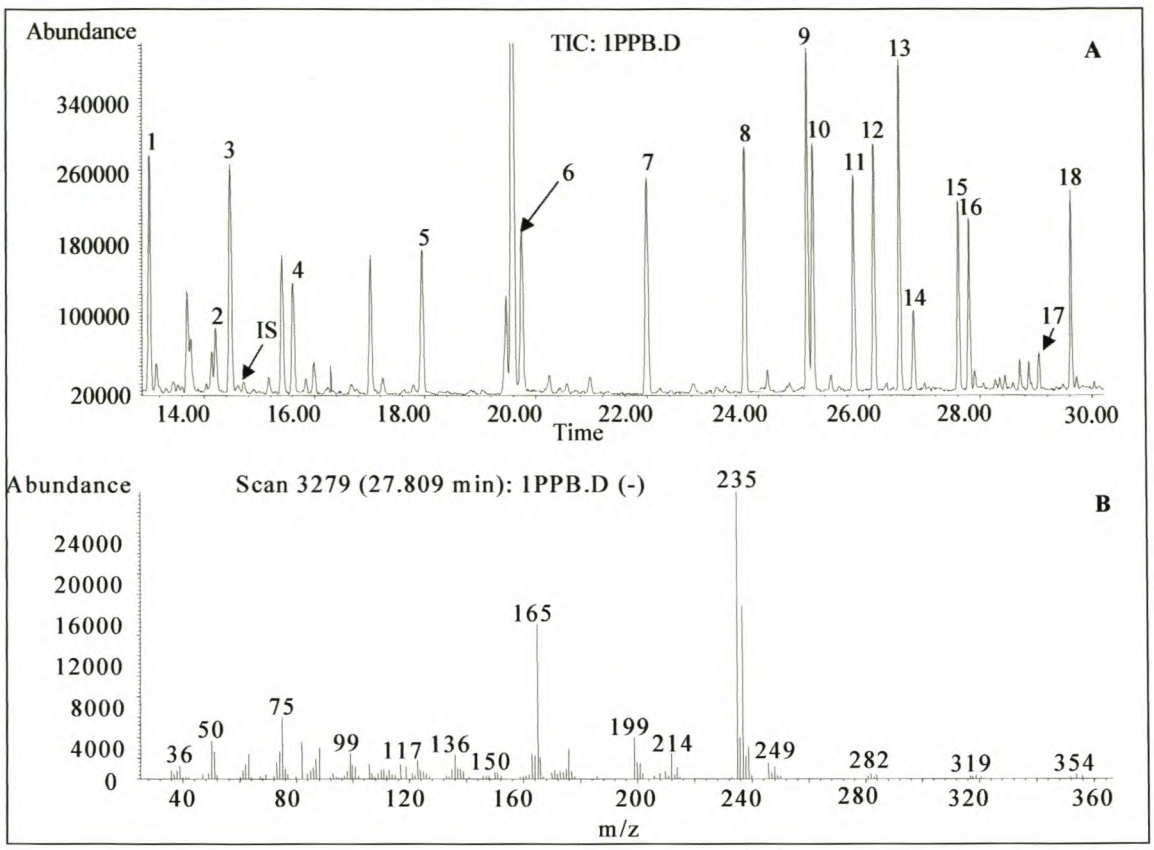


Fig 4.13. Example of (A) TDS-PTV-GC-MS total ion chromatogram (TIC) of 1 ppb of the OCPs in the scan mode (B) Mass Spectrum of p,p'-DDT. **NB:** Compound identities see Fig 4.1. and Table 4.1. Concentration I.S. (pentachloronitribezene): 100 ppt.

The identification of the compounds was done from their retention times and through the MS-library. In the scan mode the LOD of the OCPs ranges from 100 ppt to 1 ppb. Since sensitivity is an issue in trace and ultra-trace analysis, another mode of operation called selective ion monitoring (SIM) was chosen. In the SIM mode three fragment ions namely the two most intense and one closer to the parent ion for each compound, were selected (Table 4.1.).

No.	Compound name	Retention time range (min.)	*m/z* ion I	*m/z* ion II	*m/z* ion III
1	α-BHC	12.3-13.6	109	181*	219
2	β-BHC	14.1-14.39	109*	181	219
3	γ-BHC	14.39-14.66	109	181*	219
I.S.	Pentachloronitrobenzene	14.66-14.97	142	237*	295
4	δ-BHC	15.25-16.00	109*	181	219
5	Heptachlor	17.00-19.00	65	100*	272
6	Aldrin	19.00-20.80	66*	91	263
7	Heptachlorepoxide	21.2-23.00	81*	237	353
8	Endosulfan I	23.3-24.50	195*	241	339
9	Dieldrin	24.8-25.04	79*	263	318
10	p,p'-DDE	25.04-25.20	176	246*	318
11	Endrin	25.60-26.00	81*	263	281
12	Endosulfan II	26.00-26.40	195*	237	339
13	p,p'-DDD	26.50-26.75	165	199	235*
14	Endrin aldehyde	26.75-27.00	67*	250	345
15	Endosulfan Sulfate	27.52-27.80	229	272*	387
16	p,p'-DDT	27.80-28.20	165	199	235*
17	Endrin ketone	28.80-29.40	67*	250	317
18	Methoxychlor	29.40-30.00	113	152	227*

Table 4.1. The selected three ions for each of the OCP's and their elution in order and time. * The main fragment ion.

Using the SIM mode a much-improved sensitivity with LODs that range between 1 ppt and 100 ppt, and LOQs between 5 ppt and 1 ppb for all the OCPs (Chapter V) was obtained. This shows that the SIM mode is much more sensitive than the scan mode. Fig 4.14 shows an analysis at 1 ppb and an inlay of 5 ppt using SIM.

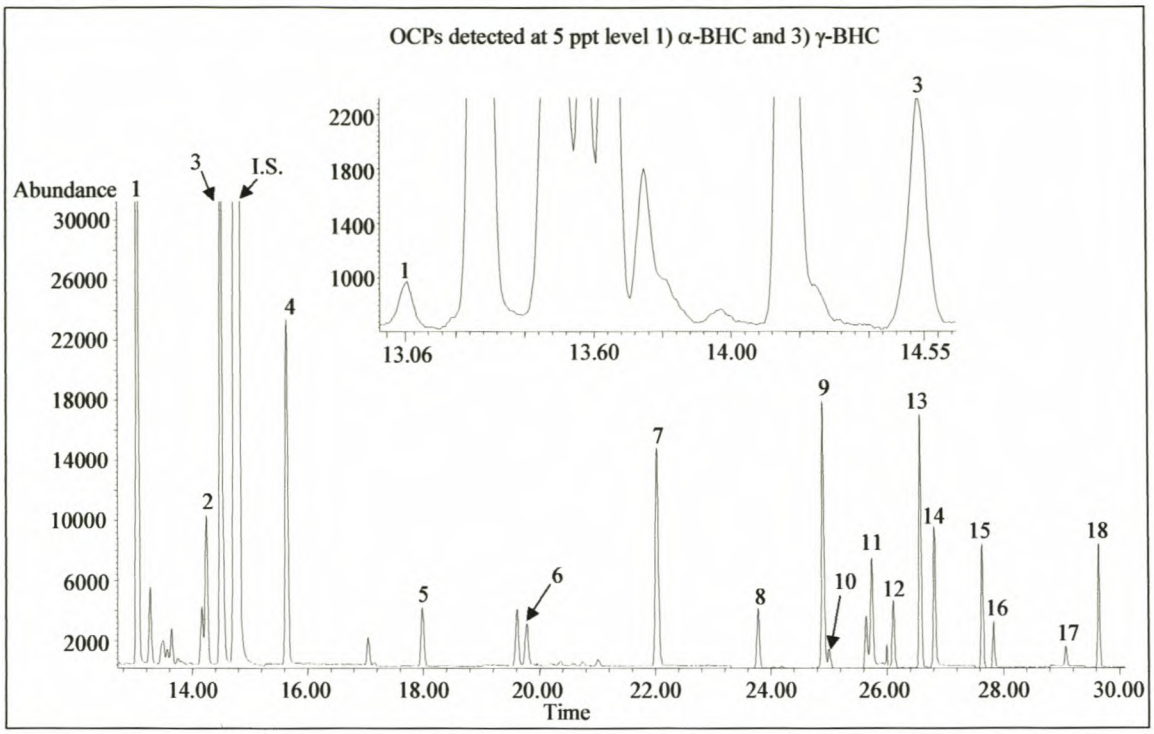


Fig 4.14. SBSE-TDS-GC-MS extracted ion chromatogram (EIC) at three *m/z* for each OCP at 1 ppb and an inlay of 5 ppt. **NB:** Compound identities see Fig 4.1.

The repeatability of the method in terms of %RSD was found to range between 0.3% to 14.4%. The linearity of the SBSE method was good with a linear range of 1:5000 with correlation coefficients greater than 0.99 for all the analytes as can be seen in Fig 4.15 for heptachlorepoxide.

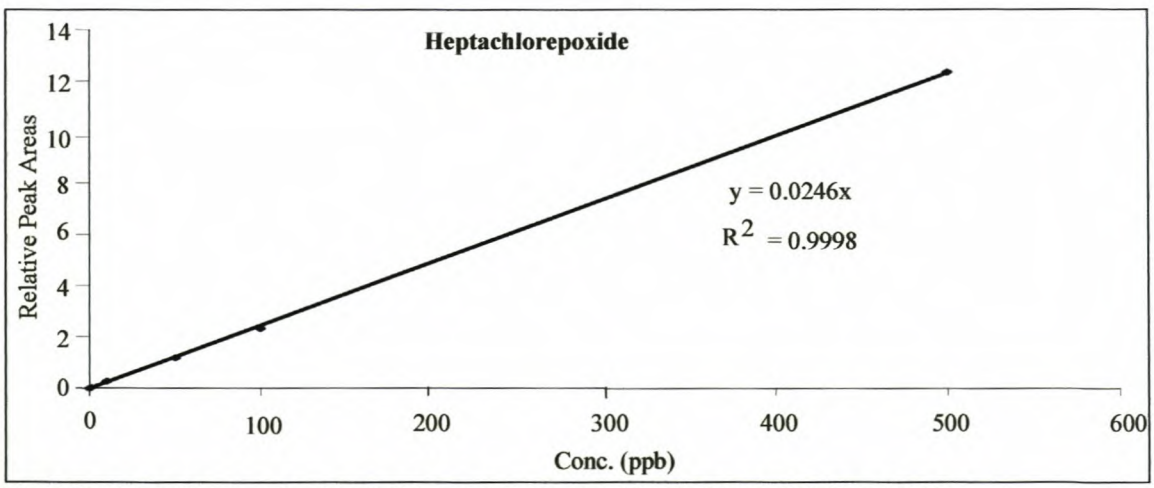


Fig 4.15. Calibration graph of heptachlorepoxide analyzed by SBSE-TDS-PTV-GC-MS.

The method was also evaluated with real contaminated water samples collected from Stellenbosch farmland (Fig 4.16). The shifts in baseline are related to the varying ions selected in the SIM mode in the different time frames.

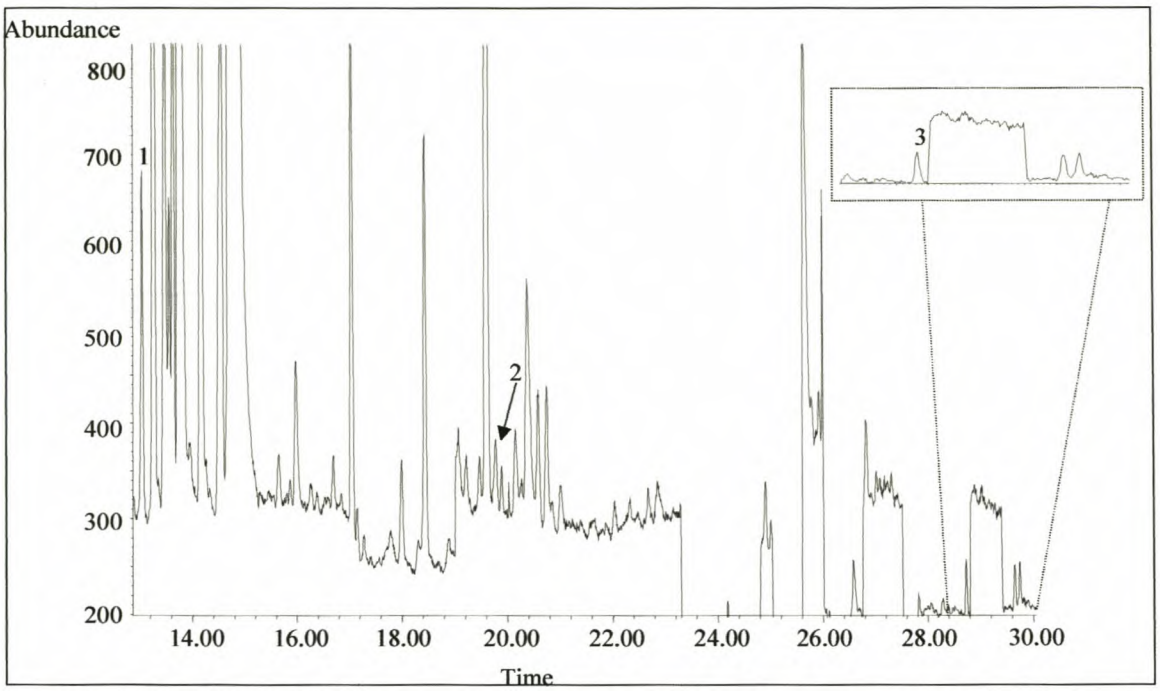


Fig 4.16. SBSE-TDS-GC-MS SIM chromatogram from contaminated water sample at three *m/z* values for each OCP 1) α-BHC = 8 ppt 2) aldrin = 40 ppt 3) p,p'-DDD = 11 ppt.

The confirmation of the compounds was done by comparison of the fragment ions of the analytes in the water sample to those selected ions for SIM mode (Table 4.1.), from the library of the GC-MS software and their retention time.

In some cases liquid desorption (LD) of the stir bar can be used as an alternative to thermal desorption. In this work SBSE-LD in combination with GC-ECD using hexane as a liquid desorber was therefore briefly investigated.

4.5.4. SBSE-LD procedure

SBSE-LD was performed by placing a specific concentration of pesticide and I.S. (pentachloronitrobenzene) in a 15 ml glass vial. The solvent (hexane) was evaporated under N_2. Deionized water (3 ml) was added to the vial and sonicated for 10 min. A new 0.5 mm film thickness PDMS coated stir bar, which was conditioned at 300 °C under N_2 flow was put in the vial and stirred for 30 min. at 900 rpm. The stir bar was dried using tissue paper and desorbed while sonicated in 1 ml hexane for 10 min. The stir bar was removed and the hexane evaporated under argon followed by re-dissolving in 100 µl hexane. A 1 µl aliquot of the re-dissolved sample was injected into the GC-ECD system and analyzed. Although this approach was successful it was obviously less sensitive than SPME-GC-ECD and was therefore not chosen as a method for routine analysis (see Fig. 4.14).

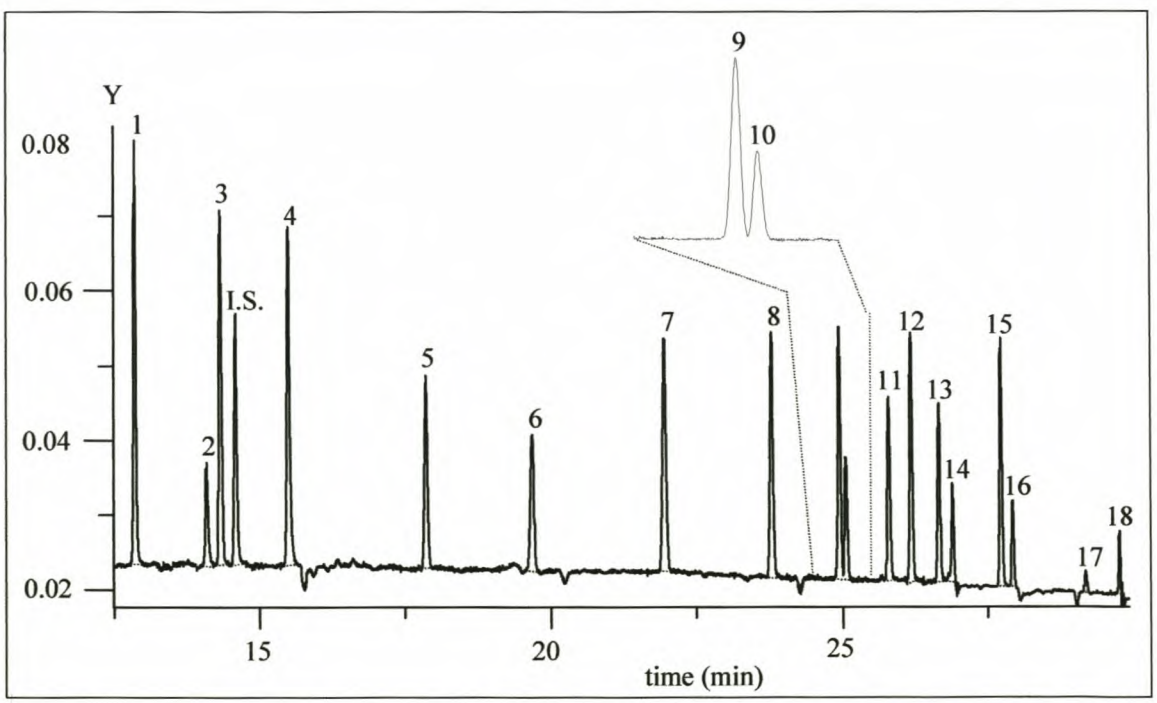


Fig 4.17. Sample chromatogram of 1 ppb OCP standard solution analyzed by SBSE-LD-GC-ECD. **NB:** Compound identities see Fig 4.1.

Besides the obvious loss in sensitivity due to the reduced concentration factor related to re-dissolving in the hexane, an additional factor to be taken into consideration is the incomplete desorption from the apolar PDMS in the stir bar to the hexane, which could further reduce the sensitivity.

A remark to be made in this study is that, although the imported standard mixture containing 2000 µg/ml of each analyte dissolved in toluene:hexane (50:50, v/v) was a mixture of only 17 OCPs, in all the experiments, 18 OCP peaks were obtained (excluding I.S.). After many attempts to adjust the injector, the detector and the oven conditions such as to resolve the degradation problem, it could only be concluded that the degradation of endrin to its isomer endrin ketone occurred in the originally purchased standard mixture.

References

1. A. Zapf, R. Heyer and H. Stan. *J. Chromatogr. A*, **1995**, 694, 453-461.
2. R. Doong, S. Chang and Y. Sun. *J. Chromatogr. A*, **2000**, 879, 177-188.
3. J. Beltran, F.J. López and F. Hernández. *J. Chromatogr. A*, **2000**, 885, 389-404.
4. P. Sandra, B. Tienpont and F. David. *J. Chromatogr. A*, **2003**, 1000, 299-309.
5. F. David, A. Tredoux, E. Baltussen and P. Sandra. *23rd International Symposium on Capillary Chromatography-Riva del Garda*, Italy, **2000**.
6. E. Baltussen, P. Sandra, F. David and C. Cramers. *J. Microcol. Sep.* **1999**, 11, 737-747.
7. H.A. Baltussen. *New Concepts of Sorption Based Sample Preparation for Chromatography*. PhD Dissertation, Eindhoven University of Technology, The Netherlands, **2000**.

Chapter V

Comparison of the developed methods and analysis of Eritrean water samples

In this chapter the widest possible comparison of the figures of merit of the different methods developed is given. This is followed by the analysis of a series of Eritrean water samples (Central zone or Zoba Maekel) with the most preferred method.

5.1. μLLE-GC-ECD, SPME-GC-ECD and SBSE-TDS-PTV-GC-MSD

The different sample preparation methods developed in this study (Chapter IV) were all found to be acceptable methods for both the qualitative and quantitative analysis of OCPs in natural waters. All the methods were found to be able to detect the maximum allowable level of OCPs set by the regulatory agencies, which is 100 ppt in water [1]. However, a selection between the methods is required in terms of their relative performance. In this chapter the practical viability of the various sample preparation methods developed is compared.

5.1.1. μLLE-GC-ECD

As a simple, inexpensive, and fast technique, μLLE combined with GC-ECD has been successfully applied for the analysis of OCPs in natural waters with sufficient sensitivity for almost all compounds. Since only 200 μl of organic solvent (hexane) is used for the extraction, it can also be classified as an environmentally friendly sample preparation method.

An advantage of μLLE-GC-ECD is that almost all of the OCPs show the same peak intensities for injection of an extracted sample or for analysis of a pure standard of the same concentration in hexane. This implies that no discrimination of the analytes is occurring and that, hence, the recovery is close to 100%. As can be seen in Table 5.1. the sensitivity, linearity and repeatability of injections are more than acceptable.

Peak No.	*Compound name*	LOD (ppt)	LOQ (ppt)	%RSD at 10ppb (n=4)	Average retention time (min)	R^2
1	α-BHC	<100	100	6.98	12.853	0.9802
2	β-BHC	<100	400	7.38	14.079	0.9970
3	γ-BHC	<100	100	2.92	14.323	0.9912
4	δ-BHC	<100	100	8.27	15.496	0.9987
5	Heptachlor	<100	100	4.20	17.877	0.9874
6	Aldrin	<100	100	7.48	19.698	0.9944
7	Heptachlor epoxide	<100	100	11.20	21.977	0.9888
8	Endosulfan I	<100	100	11.88	23.800	0.9913
9	Dieldrin	<100	100	11.75	24.956	0.9891
10	p,p'-DDE	<100	100	12.87	25.063	0.9981
11	Endrin	<100	100	12.99	25.802	0.9949
12	Endosulfan- II	<100	100	14.92	26.185	0.9961
13	p,p'-DDD	<100	100	12.45	26.651	0.9984
14	Endrinaldehyde	<100	100	16.88	26.891	0.9985
15	Endosulfan sulfate	<100	100	15.01	27.718	0.9962
16	p,p'-DDT	<100	400	10.71	27.917	0.9980
17	Endrin ketone	100	1000	9.50	29.150	0.9962
18	Methoxychlor	100	1000	18.79	29.724	0.9987

Table 5.1. Showing the LODs, LOQs, regression coefficient (r^2), and % RSD of the technique μLLE-GC-ECD using Ar/CH_4 as a make up gas.

The data obtained by this method were within the acceptable ranges for all OCPs and were consistent with literature values [2]. The method achieved a good linear range of 1:500 and a correlation coefficient of higher than 0.99 in most cases.

5.1.2. SPME-GC-ECD

One of the attractive features of the determination of OCPs using SPME combined with GC-ECD is the simple methodology and the fact that no solvent is required [3]. A drawback is that discrimination of some compounds was observed. For example, the signal of β-BHC showed a decreased intensity as compared to its isomers (α-BHC, γ-BHC and δ-BHC). Nevertheless the use of SPME in combination with GC-ECD appeared to be very suitable for trace and ultra-trace analysis. Detection could be performed down to the 1 ppt level for most of the compounds. Since OCPs are generally

found in a very low concentration in the environment and particularly in natural water systems, SPME followed by GC-ECD is a suitable and sensitive technique for both qualitative and quantitative analysis. Table 5.2. summarizes the LODs, LOQs, repeatability, linearity and average retention times of each compound.

Peak No.	*Compound name*	LOD (ppt)	LOQ (ppt)	%RSD of 500 ppt (n=4)	Average retention time(min)	R^2
1	α-BHC	1	50	6.81	12.822	0.9861
2	β-BHC	100	500	11.90	14.062	0.9993
3	γ-BHC	1	50	7.63	14.300	0.9985
4	δ-BHC	5	50	12.00	15.487	0.9998
5	Heptachlor	1	5	6.45	17.862	0.9979
6	Aldrin	5	50	11.56	19.669	0.9874
7	Heptachlor epoxide	1	5	5.38	21.956	0.9901
8	Endosulfan I	1	5	5.53	23.796	0.9909
9	Dieldrin	1	5	5.50	24.958	0.9781
10	p,p'-DDE	5	10	23.00	25.068	0.9885
11	Endrin	5	10	7.24	25.808	0.9893
12	Endosulfan- II	1	5	6.02	26.191	0.9911
13	p,p'-DDD	1	5	12.01	26.662	0.9981
14	Endrinaldehyde	5	10	8.76	26.898	0.9998
15	Endosulfan sulfate	1	5	7.01	27.730	0.9978
16	p,p'-DDT	5	10	38.39	27.929	0.9959
17	Endrin ketone	5	10	7.66	29.179	0.9961
18	Methoxychlor	10	10	4.48	29.760	0.9961

Table 5.2. Showing the LODs, LOQs, regression coefficients (r^2), and % RSD of SPME-GC-ECD using Ar/CH_4 as a make up gas.

The results obtained are comparable to those recently reported for OCP analysis from fish tissue using SPME-GC-ECD [4]. The analysis time per sample was around 2 h, which is longer compared to μLLE-GC-ECD, but the high sensitivity and the solvent-free aspect of the SPME method outweigh this disadvantage.

The high variation of p,p'-DDT and p,p'-DDE are related to degradation of p,p'-DDT into p,p'-DDE and p,p'-DDD [5]. For the rest of the OCPs the variations are within the acceptable range with an average % RSD of 10.4. The linearity of the method was found to be good with a correlation coefficient higher than 0.99 in most cases with a linear

range of 1:5000. This means that the SPME method showed a ten-fold improvement in linear range compared to the μLLE method.

5.1.3. SBSE-TDS-PTV-GC-MS

SBSE, like SPME, is a solvent-free sample preparation method, which mainly differs in the amount of PDMS used for extraction. As in SPME, in SBSE all the OCPs do not have the same affinity towards PDMS and as a result a variety of LODs and LOQs were obtained. In the MS scan mode it was possible to have LODs ranging from 100 ppt to 1 ppb. In the SIM mode much improved sensitivities with LODs and LOQs ranging from 1 ppt to 100 ppt and 5 ppt to 1 ppb (Table 5.3.) were obtained, respectively. Good repeatability with % RSD's ranging from 0.3% to 14% were noted. The linearity was good with a regression coefficient greater than 0.99 for all the analytes and a linear range of 1:5000. The obtained results are in every way comparable to the reported values [6].

We have to note that the MS used is not the most sensitive. New MS systems have a 10-fold increase in sensitivity but unfortunately such a system was not available for our studies. Moreover a recent development in negative chemical ionization MS approaching the sensitivity of the ECD has also been introduced [7].

Peak No.	***Compound name***	**LOD (ppt)**	**LOQ (ppt)**	**%RSD of 10ppb (n=3)**	**Average Retention time (min)**	**R^2**
1	α-BHC	5	10	6.15	13.187	0.9985
2	β-BHC	100	500	9.28	14.330	0.9988
3	γ-BHC	1	5	4.18	14.587	0.9994
4	δ-BHC	5	10	9.04	15.713	1.0000
5	Heptachlor	10	100	11.82	18.038	0.9982
6	Aldrin	5	100	4.37	19.830	0.9991
7	Heptachlor epoxide	5	10	11.90	22.118	0.9998
8	Endosulfan I	10	100	10.91	23.857	0.9995
9	Dieldrin	5	10	9.85	24.957	0.9980
10	p,p'-DDE	50	500	5.20	25.052	0.9958
11	Endrin	10	100	14.36	25.805	0.9996
12	Endosulfan- II	50	500	5.40	26.193	0.9989
13	p,p'-DDD	5	50	3.70	26.643	0.9964

14	Endrinaldehyde	10	50	3.17	26.907	0.9972
15	Endosulfan sulfate	50	100	1.74	27.720	0.9970
16	p,p'-DDT	50	100	5.55	27.873	0.9990
17	Endrin ketone	100	1000	0.34	29.070	0.9984
18	Methoxychlor	50	100	3.40	29.690	0.9963

Table 5.3. Showing the LODs, LOQs, Regression coefficient (r^2), and %RSD of the technique SBSE-TDS-PTV-GC-MS.

5.1.4. Comparison of the methods

It can be seen from the tables given above that the repeatabilities of all three methods are comparable. Moreover the linear range for the SPME-GC-ECD and the SBSE-GC-MSD methods were found to be the same. The SPME and SBSE total analysis time were, however, longer compared to μLLE (2 h for both SPME and SBSE and 1 h for μLLE). Obviously the three methods differ in sensitivity with μLLE being the least sensitive method. Table 5.4. combines the LOD and observed variations of the three methods.

Compound name	**μLLE-GC-ECD**		**SPME-GC-ECD**		**SBSE-GC-MSD**	
	LOD (ppt)	**%RSD** (n = 4)	**LOD (ppt)**	**%RSD** (n = 4)	**LOD (ppt)**	**%RSD** (n = 3)
α-BHC	<100	6.98	1	6.81	5	6.15
β-BHC	<100	7.38	100	11.90	100	9.28
γ-BHC	<100	2.92	1	7.63	1	4.18
δ-BHC	<100	8.27	5	12.00	5	9.04
Heptachlor	<100	4.20	1	6.45	10	11.82
Aldrin	<100	7.48	5	11.56	5	4.37
Heptachlorepoxide	<100	11.20	1	5.38	5	11.90
Endosulfan I	<100	11.88	1	5.53	10	10.91
Dieldrin	<100	11.75	1	5.50	5	9.85
p,p'-DDE	<100	12.87	5	23.00	50	5.20
Endrin	<100	12.99	5	7.24	10	14.36
Endosulfan II	<100	14.92	1	6.02	50	5.40
p,p'-DDD	<100	12.45	1	12.01	5	3.70
Endrinaldehyde	<100	16.88	5	8.76	10	3.17
Endosulfan Sulfate	<100	15.01	1	7.01	50	1.74
p,p'-DDT	<100	10.71	5	38.39	50	5.55
Endrin Ketone	100	9.50	5	7.66	100	0.34
Methoxychlor	100	18.79	10	4.48	50	3.40

Table 5.4. LODs and %RSD of the techniques μLLE-GC-ECD, SPME-GC-ECD and SBSE-TDS-PTV-GC-MS.

SPME and SBSE were observed to be affected by many conditions like pH, temperature, extraction time and the mode of extraction (direct or headspace) while μLLE does not. SPME and SBSE therefore require much more optimization compared to μLLE as all analyses have to be done at the same conditions, otherwise small difference (in the above conditions) might lead to a larger error. However, the small amounts of organic solvent used can still be considered as a drawback compared to the solventless extraction methods. Moreover due to the presence of the OCPs in the environment in a very low amount (sub ppt level to tens of ppt) it is often not easy to successfully analyze OCPs both qualitatively and quantitatively using μLLE-GC-ECD.

One of the main problems with μLLE observed in this study was easy contamination of the microsyringe used. The OCPs tend to accumulate in the glue holding the syringe needle into the barrel.

In SPME no significant contamination problems were encountered because the fiber can easily be cleaned, avoiding carry-over to the subsequent run. However, a variation in retention times was often observed due to the manual injection. However, the retention times were reproducible in the case of the SBSE approach because the TDS, PTV and GC-MS conditions are programmed in a way that the desorption, injection and analysis, respectively, can be done completely automated.

It has to be mentioned that discrimination of some compounds (Appendix A, Fig. 5) was observed with SPME and SBSE. This results from the different physical and chemical properties of each pesticide affecting its affinity to the PDMS. For example β-BHC was hardly detected at the lowest detection limit of SPME, but with μLLE almost all of the compounds could be detected with similar intensities.

The detector response was also one of the factors affecting the LOD of each compound. The ECD detector response is related to the number of halogens and their respective orientation in each compound. For example, although the number of chlorine atoms in the BHC-isomers is the same, the response by the detector is different for each isomer.

The cost effectiveness is also an important criterion in environmental samples analysis. Although stir bars are cheap to buy, the SBSE-TDS-GC-MSD system is much more expensive compared to SPME-GC-ECD.

Taking all above-mentioned differences and similarities of the obtained results and methods into account, it can be stated that SPME-GC-ECD and SBSE-TDS-GC-MSD show a similar performance and allow detection down to 1 ppt level. This is mainly because the less sensitive character of the MS is compensated for by the large amount of PDMS on the glass-covered magnetic stir bar.

Even though the maximum tolerable amount of the OCPs in natural drinking water is set by regulatory agents at 100 ppt [1], which can be achieved by both methods, in the environment OCPs are generally (and fortunately) found in lower concentrations, which require the most sensitive techniques to observe them. In addition to the 100 ppt norm for the OCPs it should also be noted that the sum of all pesticides should not exceed 0.5 μg/l (500 ppt) in drinking and ground water. Hence, a technique like SPME-GC-ECD or SBSE-GC-MS seems to impose itself. It is clear that the most powerful system for the analysis of this type of compounds would be a combination of SBSE-TDS-PTV-GC-ECD and MSD with a splitted flow at the end of the column allowing simultaneous detection with the ECD and unambiguous identification by the MS. But it can be argued that the costs of such a system outweigh the required information for OCPs analyses. Another possibility for a better analysis with a high sensitivity is negative chemical ionization MS.

Therefore due to its sensitivity, cheapness, simplicity and general availability, it was decided to analyse the water samples collected from Eritrea using SPME in combination with GC-ECD.

5.2. Analysis of Eritrean water samples using the SPME-GC-ECD technique

The area selected for this study is the central zone (zoba maekel), which includes Asmara, the capital city of Eritrea, and surrounding areas (Fig 5.1.). This area uses surface water as the main source of water for domestic use. All the sources are reservoirs and dams such as the Mai-Nefhi dam, Adi-Nifas dam, Tokor dam, Mai-Bahria dam and the Mai-Surwa dam. The two main water treatment plants are Mai-Nefhi and Adi-Nifas but the others like Mai-Bahria are small dams providing water for certain parts of Asmara by performing a primary treatment like adding chlorates as disinfectants. In the Mai-Nefhi and Adi-Nifas water treatment plants the main classes of chemicals used are phosphates or sulfates as coagulants or precipitants of suspended particles and chlorates as disinfectants.

Fig 5.1. Geographical map of Eritrea. (Source: UN consolidated appeal for Eritrea, January 2001, p.3)

A total of 26 representative water samples were collected from the area selected for this study. All samples were stored at –4 °C until analysis. The pH, conductivity, and temperature of each sample were measured during sampling. Moreover, to have a clear idea of the sites location, the position of each sampling site was measured in terms of longitude, latitude, and altitude using a global positioning system (GPS). Both the longitude and the latitudes are in Universal Transverse Mercator (UTM) while the altitude is in meters above sea level (Table 5.5.).

Sample No.	Sampling site name	Measured parameters during sampling			Sampling Position		
		pH	EC (µs)	Temp. (°C)	Longitude (UTM)	Latitude (UTM)	Elevation (meter)
1	Adi-Nifas WTP1	7.00	216	18.5	1701781	0491363	2393
2	Adi-Nifas WTP1	7.10	217	16.9	1701657	0491314	2404
3	Adi-Nifas WTP1	6.82	211	20.2	1701657	0491281	2367
4	Adi-Nifas WTP1	8.35	270	17.00	1701810	0491160	2374
5	Adi-Nifas dam	7.10	215	19.10	1702204	04931221	2363
6	Adi-Nifas WTP2	6.55	204	18.70	1702147	0491510	2386
7	Tokor dam	6.68	201	18.80	1706733	0482350	2172
8	Mai-Surwa dam	7.39	189	19.60	1702034	0489127	2329
9	Mai-Nefhi WTP	6.93	256	17.10	1686290	0476492	2164
10	Mai-Nefhi WTP	6.86	275	17.60	1686279	0476486	2177
11	Mai-Nefhi dam	6.97	266	16.90	1686201	0476922	2198
12	Kutmowli'e tap water	6.92	269	18.80	1686374	0481343	2258
13	Adi-Shakha dam	8.27	176.40	20.10	1712728	0488584	2407
14	Beleza PP	8.76	207	21.50	1704384	0491458	2413
15	Hazhaz reservoir tank	6.74	217	16.40	1696983	0492848	2368
16	Lageto-dam #1 (Mai-Bahria)	7.03	408	16.00	1695956	0495107	2363
17	Lageto-dam #1 (Mai-Bahria)	7.60	502	16.60	1695956	0495107	2363
18	Lageto-dam #2 (Mai-Bahria)	7.15	115.1	17.30	1696101	0495430	2378

19	Asmara Cigarette factory tap water	6.92	211	19.20	1695164	0493525	2364
20	Meloti beer factory (waste)	7.51	1751	20.30	1693699	0493208	2343
21	Tap water close to beer factory	6.68	259	15.10	1693693	0493064	2335
22	Sembel Pump-Station	6.73	272	19.40	1691498	0489289	2331
23	Sembel Pump-Station	6.81	269	18.5	1691479	0489283	2336
24	Expo Pump-Station	6.63	270	19.60	1693212	0490902	2323
25	Mai-Bella (well)	6.90	3000	19.50	1696222	0491023	2323
26	Geza-Banda reservoir tank	6.74	256	18.40	1694644	0493599	2332

Table 5.5. The pH, electrical conductivity (EC), temperature, site name and site location of the Eritrean water samples.

In most of the sites more than one sample was taken because of suspected differences in contamination in the water system depending on the location. From the 26 Eritrean water samples (EWS) 7 of them showed to be contaminated with some of the OCPs. The common OCPs detected in the EWS are α-BHC, heptachlor, heptachlorepoxide, endosulfan I, p,p'-DDE, endosulfan II, p,p'-DDD, endosulfan sulfate, and p,p'-DDT.

The water samples that are contaminated with the OCPs are EWS2, EWS9, EWS12, EWS17, EWS20, EWS21, and EWS24. The identities of the OCPs in the Eritrean water samples were confirmed by comparing their retention times with those of OCPs standards and by spiking with standards.

Although the water samples EWS1 (water that enters to the plant from the main source), EWS2 (after treatment only by a sedimentation process), EWS3 (finally treated and distributed to the people for drinking) and EWS4 (waste of the plant) were collected from the same water treatment plant Adi-Nifas (WTP1), however, only EWS2 showed to have four of the OCPs (Fig. 5.2.) in small amounts. The OCPs found in the EWS2 are:

heptachlorepoxide (5 ppt), endosulfan II (4 ppt), p,p'-DDD (5 ppt), and endosulfan sulfate (5 ppt).

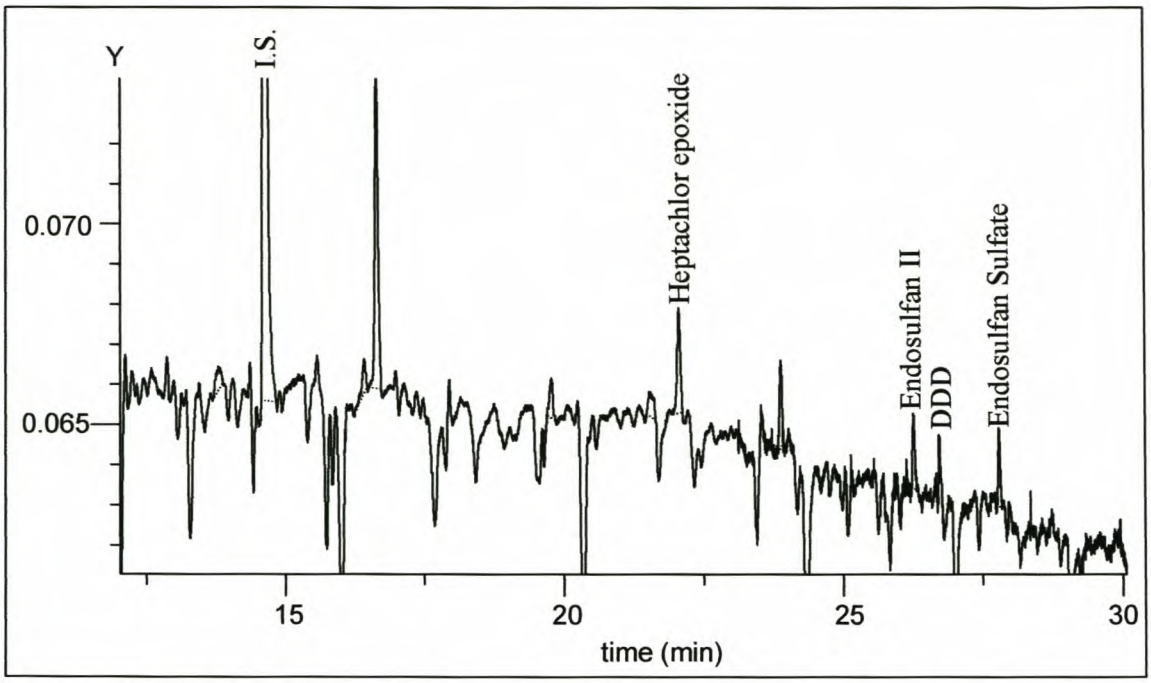


Fig 5.2. SPME-GC-ECD chromatogram of sample EWS2. The pesticides detected were heptachlor epoxide (5 ppt), endosulfan II (4 ppt), p,p'-DDD (5 ppt), endosulfan sulfate (5 ppt).

Samples EWS9 and EWS10 were collected from the Mai-Nefhi water treatment plant but only in EWS9 OCPs were detected. EWS9 was taken from the totally treated water and EWS10 was collected from the water treated by sedimentation. The OCPs detected in the sample EWS9 are (Fig 6. Appendix B): α-BHC (9 ppt), heptachlor (15 ppt), endosulfan II (5 ppt), and p,p'-DDT (112 ppt). As can be seen the amount of p,p'-DDT is above the maximum tolerable amount of pesticides in drinking water [1].

EWS12 was sampled from a village called Kutmowli'e directly from the tap water. The source of the water for this village is from Mai-Nefhi water treatment plant and it is situated between the water treatment plant and the capital city Asmara. Not surprisingly some of the OCPs detected in EWS9 were also observed here. The pesticides quantified in this water sample are: heptachlor (10 ppt) and p,p'-DDT (41 ppt).

EWS17 was collected from Mai-Bahria, a small dam situated in the eastern part of the city called Lagetto near a shoe factory "Dellux". It provides water for certain parts of the city like the Deposito area. EWS16 and EWS17 were taken from the same place with the difference that the latter is taken from the part of the water disinfected by chlorination. In the EWS17 sample endosulfan sulfate (31 ppt) was detected.

EWS20 was collected from of the Melloti Beer factory. The sample is the waste water of the factory collected during the cleaning day of the whole factory. In this water sample heptachlor with a concentration of 14 ppt was quantified. EWS21 was collected from tap water in a place called Setanta'oto situated next to the beer factory. Three OCPs were detected in this tap water sample: heptachlor (6 ppt), endosulfan II (3 ppt), and p,p'-DDT (24 ppt).

The last Eritrean water sample, which showed to have some of the OCPs, is EWS24. It was sampled from the Expo-pump station, which comes from the Mai-Nefhi water treatment plant after treatment and is transferred to the houses via this station for domestic use. Again the water originating from this source showed to contain: α-BHC (7 ppt) and p,p'-DDT (67 ppt).

The quantification of the OCPs in the Eritrean water samples given above for each sample was done using the internal calibration method. Table 5.6. shows the detected OCPs and their concentration in the Eritrean water samples.

	Concentration (ppt)																	
EWS No.	α-BHC	β-BHC	γ-BHC	δ-BHC	Heptachlor	Aldrin	Heptachlorepoxide	Endosulfan I	Dieldrin	p,p'-DDE	Endrine	Endosulfan II	p,p'-DDD	Endrinaldehyde	Endosulfan Sulfate	p,p'-DDT	Endrin keton	Methoxychlor
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	**5**	0	0	0	0	**4**	**5**	0	**5**	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	**9**	0	0	0	**15**	0	0	0	0	0	0	**5**	0	0	0	**112**	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	**10**	0	0	0	0	0	0	0	0	0	0	**41**	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**31**	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	**14**	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	**6**	0	0	0	0	0	0	0	0	0	**3**	**24**	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	**7**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**67**	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5.6. The OCPs detected in the Eritrean water samples with their concentration in ppt. **NB:** zero (0) indicates not detected.

Although these compounds have been banned for many years, they can still be detected in the Eritrean environment as can be seen from the results given above. The chromatograms for the rest of the Eritrean water samples that showed to have OCPs are given in Appendix B.

The results from the analyses of water samples collected from the selected part of Eritrea illustrate that the concentration of most of the OCP residues detected in almost all of the water samples are below the maximum allowable concentration of 100 ppt value set by the European Union (EU) for the protection of human health [1]. Only the elevated level of 112 ppt p,p'-DDT detected in EWS9 gives cause for concern considering its domestic use. Although the concentration of p,p'-DDT in the other water samples is below the

norm its amount is still much higher than the rest of the OCPs detected in the samples. All this information seems to indicate that the Mai-Nefhi water treatment plant suffers from some degree of DDT contamination. The source of this contamination is hard to identify but it is probably related to the intensive use of p,p'-DDT spray in households as an insecticide and for killing mosquitoes for malaria control [8].

These results are rather surprising considering that the Eritrean environment was expected to be exempt from OCPs contamination compared to other countries because of its less developed nature in terms of industry and agriculture.

References

1. *EEC Drinking Water Guideline*, 80/779/EEC, EEC No. L229/11-29, European Economic Community, Brussels, 30 August **1980**.
2. A. Zapf, R. Heyer and H. Stan. *J. Chromatogr. A*, **1995**, 694, 453-461.
3. L. Anelli, F. Munari, A. Trisciani and J. R. Castanho. *Determination of Water Contaminants at Trace Levels Using Large-volume Sampling Techniques: Comparison of LVPTVI, LVOCI, and SPME.* Environmental Application Note, **May 2001**. http://www.iscpubs.com/articles/aln/n0105ane.pdf.
4. N. Fidalgo-Used, G. Centineo, E. Blanco-González and A. Sanz-Medel. *J. Chromatogr A*, **2003**, 1017, 35-44.
5. S. Magdic and J. B. Pawliszyn. *J. Chromatogr. A*, **1996**, 723, 111-122.
6. P. Popp, C. Bauer, P. Keil and L. Wennrich. *J. Sep. Sci.* **2003**, 26, 961-967.
7. X. Xiao, D. V. McCalley, and J. McEvoy. *J. Chromatogr. A*, **2001**, 923, 195-204.
8. Government of Eritrea. *National Environmental Management Plan for Eritrea*, Drukerei Schwenk and Co. GmbH, Frankfurt, Germany, **1996**.

Chapter VI

Analysis of organochloro-pesticides in soil and sediment samples

6.1. Introduction

It has been estimated that approximately half of the overall soil sources have been degraded as a result of industrialization and the quality of soil has a great impact on natural water quality [1].

Pesticide analyses in soil samples are challenging because of the low concentration level and the large number of interferences originating from compounds such as: fats, waxes and elemental sulfur (S_8). Moreover, pesticides stick to soils very strongly, making it even more difficult to extract them from the soil matrix [2]. Because of this matrix complexity and the need to analyze pesticides at trace and ultra-trace levels, analytical methods of high sensitivity, selectivity and resolving power are required for their determination. The general approach is the development of a powerful and selective extraction method followed by standard GC analysis similar to the ones described in the precedent chapters for water analysis.

The most common method for isolation of the OCPs investigated in this study from the soil is extraction with organic solvents, followed by extensive clean-up (Chapter-II) procedures in order to remove interferences prior to chromatographic analysis. Some of these techniques are: LLE, microwave-assisted extraction (MWAE) and Soxhlet extraction [3]. However, today these sample preparation methods are coming out of favor due to the laborious clean-up procedures with great chances of analyte loss and the consumption of high purity toxic organic solvents.

In the present work it is attempted to develop a solvent free extraction method by using SPME in an aqueous extract of soil and sediments followed by GC-ECD analysis.

6.2. Experimental

6.2.1. Reagents and materials

All reagents and materials have been outlined in section 4.1.

6.2.2. Finalized instrumental conditions

The CGC conditions have been described in section 4.2.1. Only Ar/CH_4 was used as make-up gas.

6.2.3. Optimized SPME extraction procedure

Soil samples for developing the SPME method were collected from Stellenbosch farmland and dried at 280 °C for 5 h to ensure that the samples were free of pesticides.
1 g of this pesticide-free soil was transferred into a 15 ml glass vial. The soil was spiked by adding appropriate amounts of a pesticide standards stock solution in hexane to obtain final concentrations of 1, 10, 50, 100, 500, and 1000 ng/g. 1ml of hexane was added to cover the soil and sonicated for 5 min to homogenize the slurry. The hexane was subsequently evaporated under N_2 to dryness.
To both the calibration and real soil samples, 10 ml deionised water was added followed by sonication at 20 °C for 15 min. The suspension was centrifuged for 15 min at 800 rpm, 7 ml of the clear supernatant was transferred to an empty 15 ml glass vial and direct-SPME at 20 °C followed by GC-ECD analysis was done in the same way as the analysis of the water samples outlined in Chapter IV.

6.3. Results and discussion

6.3.1. Development of the method

As the organochloro-pesticides (OCPs) under study are apolar, a PDMS coated SPME fiber was again chosen for extraction. Note that all OCPs are slightly soluble in water with a solubility ranging from 5 μg/l for p,p'-DDT to 7.3 mg/l for γ-BHC [4,5]. Also because of the slightly higher polarity of the endosulfan group type of OCPs (leading to high affinity towards the aqueous matrices) immersion or direct-SPME sampling was selected as an extraction mode [6].

The following parameters were evaluated in the optimization of the method: the effect of the amount of soil analyzed, the influence of the pH, the use of a two step or one step extraction procedure, the influence of salt addition and the possibility of headspace SPME.

The amount of soil analyzed was varied from 1 to 5 grams but the most suitable quantity was found to be 1 g.

The pH effect was evaluated by adding NaOH prior to extraction. Though giving higher extraction efficiencies, for some compounds such as endosulfan I, endosulfan II, and endosulfan sulfate, the recovery was generally decreasing. Therefore it was decided to perform the extraction without adjusting the pH.

The effect of salt addition was evaluated by adding 0.5 g of $(NH_4)_2SO_4$ to the deionized water so as to increase the ionic strength of the water thereby to enhance the extraction of the OCPs by the PDMS phase. This didn't present any improvement.

Headspace solid phase microextraction (HS-SPME) at 70 °C was evaluated to avoid exposing the fiber to dirty matrices. However, due to insufficient volatility of the compounds most pesticides were not detected.

Finally a two-step extraction procedure was used: first, leaching of pesticides from the soil with deionized water; secondly direct-SPME of the extracted residue in aqueous solution followed by GC-ECD analysis. The details of the procedures are outlined in section 6.2.3.

6.3.2. Figures of merit of the obtained method

The linearity of the method was evaluated by preparing five different concentrations of OCP calibrating standard solutions (10 ppt, 50 ppt, 100 ppt, 500 ppt, and 1000 ppt) and it was found to be linear from 50 ppt to 500 ppt, with a correlation coefficient (r^2) higher than 0.99 in almost all cases, which is comparable to literature values [5]. The calibration curves were corrected for the I.S. The concentrations above 500 ppt were found to be out of the linear range (Fig 6.1.).

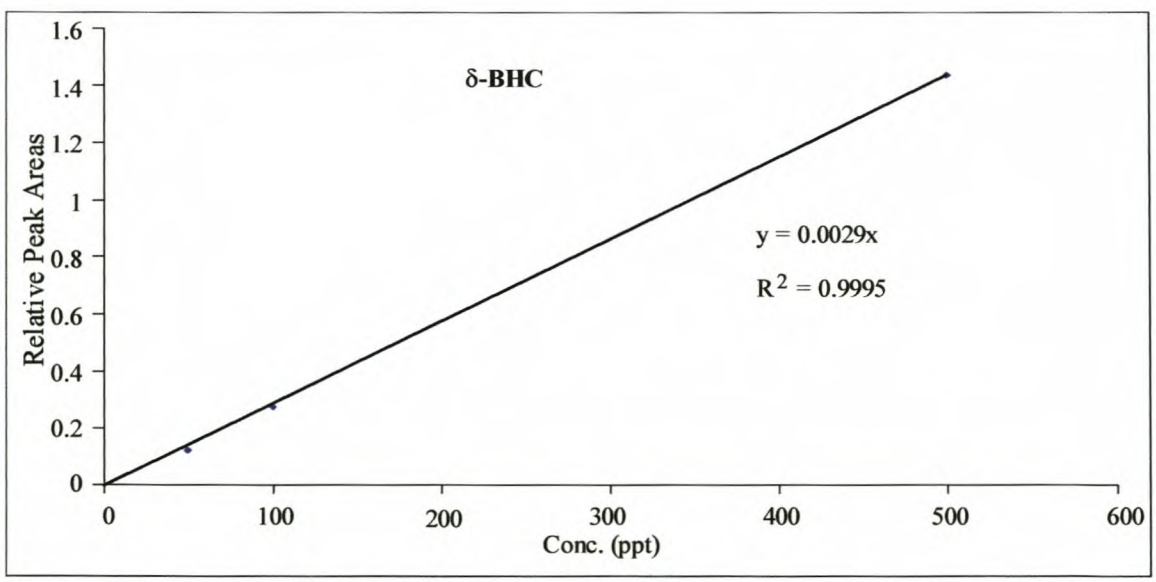


Fig 6.1. Calibration graph of δ-BHC of soil analysed by SPME-GC-ECD.

The repeatability of the analysis was evaluated by doing a series of four injections (n = 4) of 10 ppt concentration. Except for endrin, endosulfane sulfate and aldrin, which showed %RSD's of 16.1 %, 19.9 %, and 22.3%, respectively, the repeatability was found to be

between 2.4 % and 8.2 % comparable to reported literature values [5]. At a concentration of 500 ppt (0.5 ng/g) all the pesticide peaks can clearly be detected (Fig 6.2.).

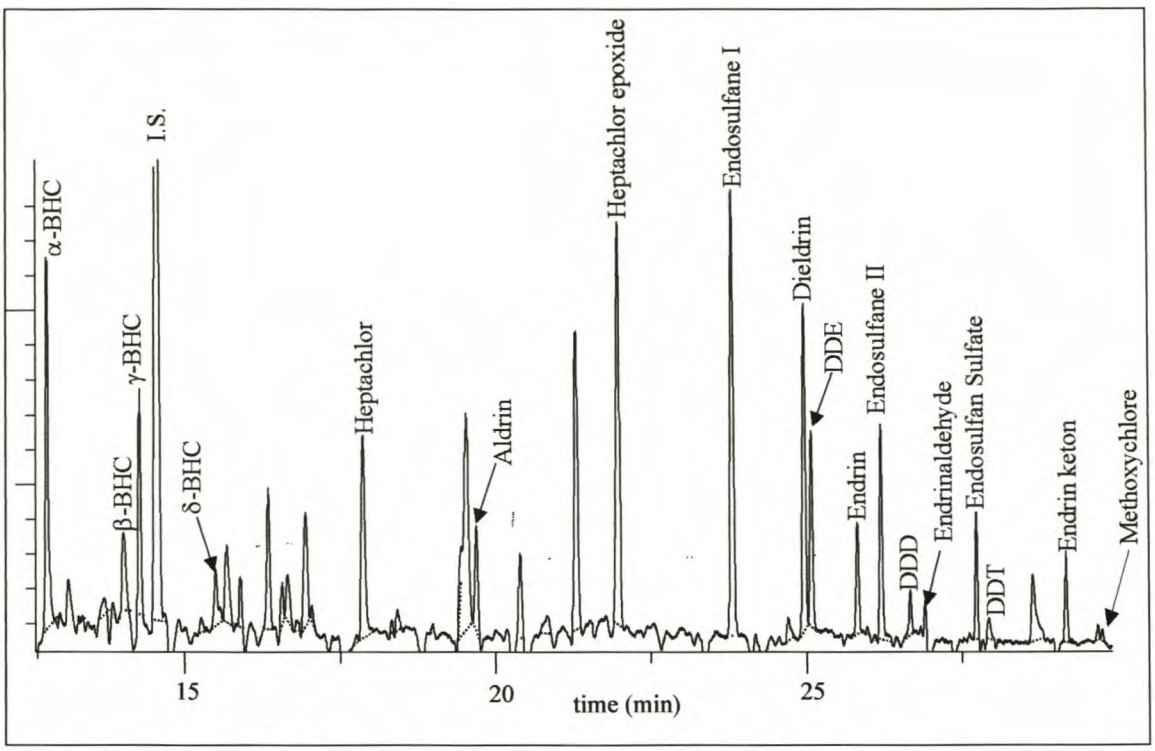


Fig 6.2. Chromatogram of SPME-GC-ECD analysis of blank soil sample spiked with 0.5 ng/g (500 ppt) of OCPs.

Due to the variation in limit of detection (LOD) of each analyte mainly depending on soil property, detector characteristics and the affinity of each compound towards the fiber, half of the peaks were found to be missing at the lowest analyzed concentration level, i.e. 1 pg/g (Appendix A, Fig. 14). The limit of detection (LOD) calculated as a signal to noise ratio 3:1 ranges from less than 1 pg/g for p,p'-DDE to 0.5 ng/g for methoxychlor which is better than reported values [2,5] and the LOQ (1:10 of the background noise) was found to range from 10 pg/g for p,p'-DDE to 1 ng/g for methoxychlor (Table 6.1.).

Peak No.	Compound name	EPA 608 MRLs (ppt)	LOD (ppt)	LOQ (ppt)	%RSD of 10ppt (n=4)	Average Retention time(min)	R^2
1	α-BHC	20	5	10	8.24	12.808	0.9992
2	β-BHC	20	10	1000	13.40	14.044	0.9999
3	γ-BHC	20	10	100	2.39	14.293	0.9989

4	δ-BHC	20	100	1000	8.12	15.473	1.000
5	Heptachlor	10	10	500	5.13	17.860	0.9971
6	Aldrin	20	10	1000	22.26	19.675	0.9966
7	Heptachlor epoxide	10	1	10	9.86	21.958	0.9979
8	Endosulfan I	20	1	10	5.68	23.794	0.9983
9	Dieldrin	20	1	10	6.88	24.951	0.9986
10	p,p'-DDE	20	<1	10	5.67	25.065	0.9965
11	Endrin	10	100	500	16.06	25.799	0.9981
12	Endosulfan- II	20	10	500	3.86	26.186	0.9981
13	p,p'-DDD	20	500	1000	6.46	26.650	0.9979
14	Endrin aldehyde	20	500	1000	11.49	26.891	0.9970
15	Endosulfan Sulfate	20	10	500	19.97	27.721	0.9984
16	p,p'-DDT	20	100	1000	2.5	27.923	0.9975
17	Endrin Ketone	-	10	500	5.16	29.172	0.9957
18	Methoxychlor	200	500	1000	-	29.720	0.9785

Table 6.1. Showing the minimum reported levels (MRLs), LODs, LOQs, Regression coefficient (r^2), slope, and %RSD. (MRLs = Minimum reported levels of target analytes).

The extraction method was also evaluated on real environmental soil samples collected from Stellenbosch farmland. In Fig 6.3., showing a chromatogram of a contaminated soil sample, γ-BHC, endosulfan I, dieldrin, endosulfan II, endosulfan sulfate and p,p'-DDT are visible. The peaks were identified with standard addition by spiking the soil with 500 ppt OCPs standard solution and re-analyzing. Quantification was done through the internal standard.

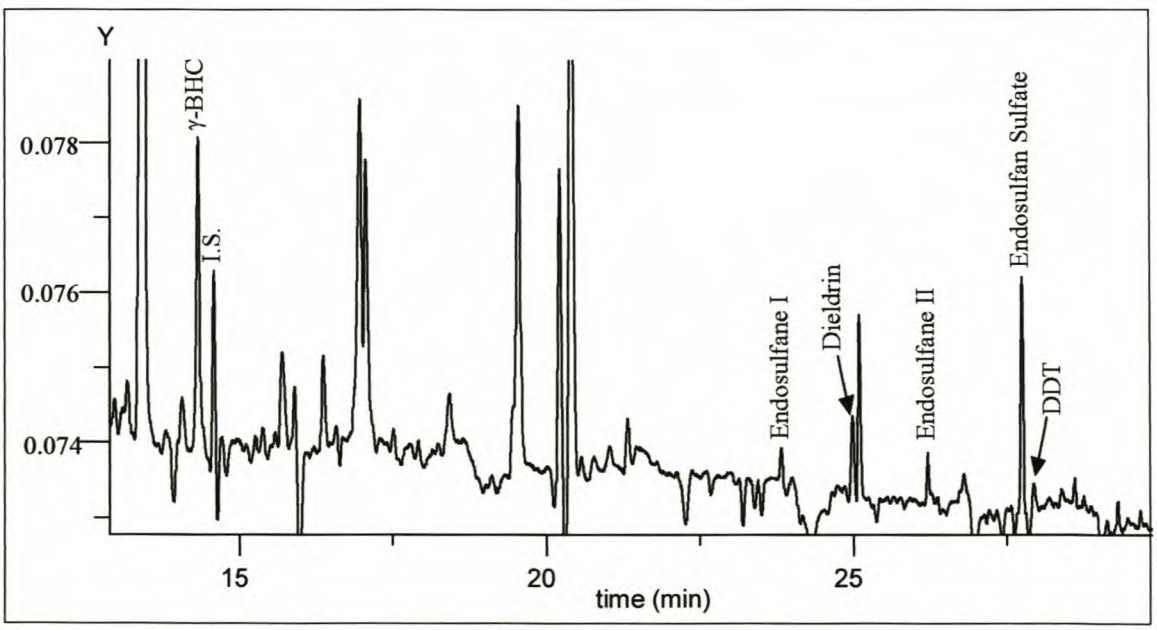


Fig 6.3. A chromatogram of a real soil sample collected from Stellenbosch farmland. The OCPs detected were: β-BHC (375 ppt), γ-BHC (367 ppt), endosulfane I (17 ppt), dieldrin (46 ppt), endosulfan II (45 ppt), endosulfan sulfate (417 ppt) and p,p'-DDT (290 ppt).

The presented method offers an alternative to established methods of pesticide analysis in soil. In this case SPME is used after transfer of the OCPs from soil to an aqueous solution. It appears that this method is a useful technique for both qualitative and quantitative analysis of OCPs in soil and sediment samples.

Even though the OCPs are classified as non-polar compounds, in this work it is seen that they can be extracted using water dependent on polarity.

Although the method developed in this chapter seems successful some reservations must be taken into account. True evaluation of the performance can only be achieved by comparison with an established liquid-liquid extraction technique. Due to time limitation this was, however, not possible within the scope of this work.

References

1. E. Macutkiewicz, M. Rompa, and B. Zygmunt. *Critical Reviews in Analytical Chemistry*, **2003**, 33, 1-17.
2. H. Prosen and L. Zupančič-Kralj. *Acta Chim. Slov.* **1998**, 45, 1-17.
3. O.S. Fatoki and R.O. Awofolu. *J.Chromatogr. A*, **2003**, 983,225–236.
4. S. Magdic and J. B. Pawliszyn. *J. Chromatogr. A*, **1996**, 723, 111-122.
5. R. Doong and P. Liao. *J. Chromatogr. A*, **2001**, 981, 177-188.
6. M.C. López-Blanco, B. Reboreda-Rodríguez, B. Cancho-Grande and J Simal-Gándara. *J. Chromatogr. A*, **2002**, 976, 293-299.

Conclusions and recommendations

Based on the outcomes of this work the following conclusions and recommendations can be made.

7.1. Conclusion

The work performed for this thesis showed that both CGC-ECD and CGC-MSD can successfully be applied for the analysis of OCPs in environmental samples. The combination of CGC with ECD detection was shown to allow the analysis of halogenated compounds with limited interferences and high sensitivity.

It was observed that the use of Ar/CH_4 (10% methane) as make-up gas in ECD improved the linear range and sensitivity compared to N_2. This could be related to a certain degree of impurities in the N_2 but it was beyond the scope of this work to perform a fundamental study concerning the performance of the ECD versus various make-up gases.

Although less sensitive, CGC-MSD has the potential of analyzing a wider range of target compounds with a clear confirmation of the analyte identities. Selective detection of analytes can be achieved using the SIM mode. However, the re-constructed ion chromatogram will often contain unwanted peaks if the environmental sample contains compounds leading to fragment ions resembling those selected. Unfortunately, the scan mode does not provide enough sensitivity for real samples. SIM improves the sensitivity but reduces considerably the qualitative information, thus increasing the risk of false positives.

All the three methods μLLE-GC-ECD, SPME-GC-ECD and SBSE-TDS-PTV-GC-MSD were shown to be usable for the analysis of OCPs in water. Comparison between the limits of quantification and the maximum residual limits showed that sensitivity of the three methods was sufficiently good to ensure a reliable determination. However, due to

the actual very low amounts of the OCPs present in the environment and due to the use of toxic organic solvents, solvent-free extraction methods were selected for further use.

SPME-GC-ECD and SBSE-TDS-PTV-GC-MSD allow the determination of target analytes quantitatively in the environment down to the 1 ppt level. Note that extractions using PDMS media are based on a sorption process instead of an adsorption process. The former offers several advantages over the latter. They also require limited sample volumes. Moreover, no organic solvents are needed thereby, risk of secondary contamination during sample handling is less. With the SBSE technique, as a result of the desorption, injection and analysis being done automatically, improved repeatabilities were obtained compared to the manually done μLLE and SPME methods.

Both SPME and SBSE sample preparation methods resulted in analyses with good LODs, LOQs, repeatabilities, linearities and a similar linear range. From these observations it can be concluded that the less sensitive behavior of the MSD can be compensated for by the large amount of PDMS phase on the stir bar. Because of the general availability, cost effectiveness, simplicity and speed, SPME in combination with GC-ECD, is more appropriate technique for the analysis of OCPs in environmental samples.

However, PDMS based extraction techniques showed some drawbacks in terms of discrimination compared to μLLE. This was clearly observed for β-BHC in the SPME method. This is related to the different partition coefficients of each analyte towards the PDMS phase.

Liquid desorption of the OCPs trapped on the stir bar, followed by splitless injection on the GC-ECD was also investigated. Although good chromatograms were obtained in this way, the approach was not further pursued due to the drop in sensitivity related to the dilution effect in the desorbing solvent.

Hence, SPME in combination with GC-ECD was chosen for the analysis of the water samples collected from Eritrea. As a result, of the 26 selected water samples from the

area, 7 of them were shown to contain OCPs. The common OCPs detected in the Eritrean water samples (EWS) were α-BHC, heptachlor, heptachlorepoxide, endosulfan I, p,p'-DDE, endosulfane II, p,p'-DDD, endosulfan sulfate, and p,p'-DDT.

From the figures of the pesticides detected in the EWS, p,p'-DDT was the most prevalent. Although four samples were collected from the same water treatment plant Adi-Nifas (WTP1), only one, which was collected from the sedimented part of the treatment plant, was shown to contain four of the OCPs. This indicates accumulation of the pesticides, present in undetectable sub ppt levels in the water, in the sediment, in which vicinity the OCPs can easily be detected. Two samples were also collected from Mai-Nefhi water treatment plant but only in one OCPs were detected (9 ppt α-BHC, 15 ppt heptachlor, 5 ppt endosulfan II, and 112 ppt p,p'-DDT). The amount of p,p'-DDT is above the maximum tolerable amount of pesticides in drinking water set by the regulatory agents.

Eritrea was expected to show very limited pollution in comparison to other countries because of its undeveloped industrial nature. It was therefore surprising to detect any of the pesticides at all in the studied samples. Possible sources of the detected pollutants can be the persistent use of p,p'-DDT in households as a general insecticide and for malaria control. Another potential source of these OCPs can be related to its agricultural use in the past.

An SPME-GC-ECD method was also developed in this study for the analysis of soil and sediment samples. This method was based on the extraction of the slightly water soluble OCPs in an aqueous media followed by SPME-GC-ECD analysis. Although the method was successful for the extraction of the OCPs from soil, due to time limitations it was not possible to investigate it in more detail.

7.2. Recommendations

Based on the overall results obtained in this study the following recommendations can temptatively be made:

- Since it has been shown that Stellenbosch farmland contains some of the OCPs, a broad survey of the presence of OCPs and other endocrine disrupting chemicals in South Africa seems to impose itself.

I believe this work will have some contribution to give some ideas on the status of the Eritrean environment. This is the first study in trace analysis and particularly pesticide analysis in Eritrea and it showed clearly the presence of some OCPs in various areas. The following recommendations can, hence, be made:

- A conducive environment for professionals, concerned individuals and organizations should be created for an open discussion regarding the status and safety of the Eritrean environment and to propose possible solutions.

- A national environmental assessment should be made particularly on the presence of OCPs with the emphasis on the land areas with a high incidence of malaria and high concomitant spraying of p,p'-DDT.

- Regulation should be strengthened to avoid the use of such hazardous pesticides for domestic purposes.

- Globally more research should be performed to offer another viable way of controlling malaria instead of using p,p'-DDT.

Appendix A

Selected Chromatograms

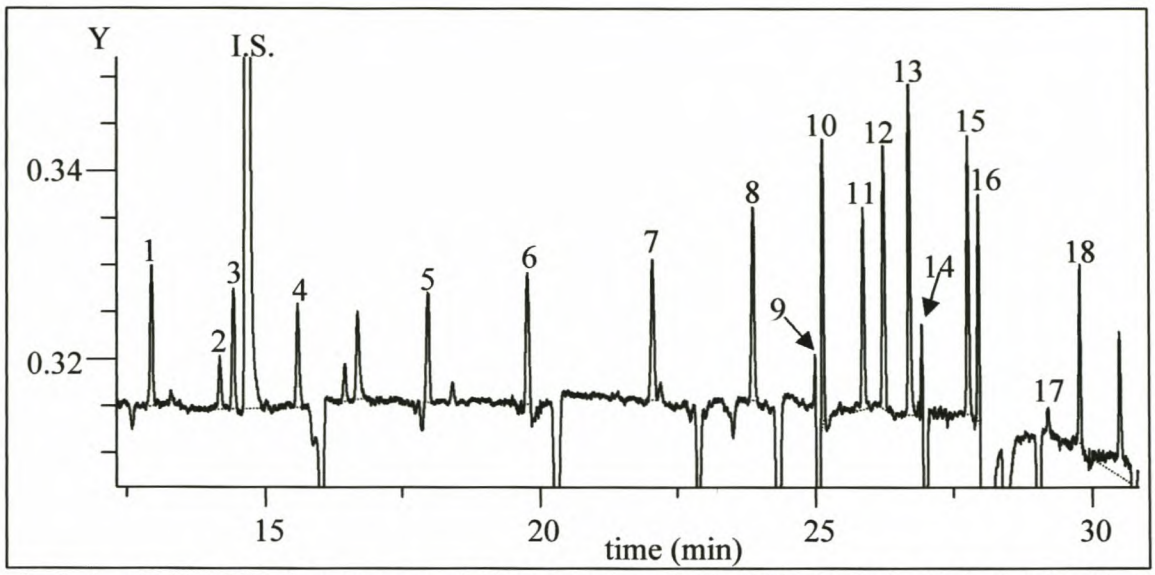


Fig 1. 1 ppb GC-ECD chromatogram of direct injection of 17- OCP standards obtained using N_2 as make-up gas. 1. α-BHC, 2. β-BHC, 3. γ-BHC, I.S (Pentachloronitrobenzene), 4. δ-BHC, 5. Heptachlor, 6. Aldrin, 7. Heptachlorepoxide, 8. Endosulfan I, 9. Dieldrin, 10. DDE, 11. Endrin, 12. Endosulfan II, 13. p,p'-DDD, 14. Endrin aldehyde, 15. Endosulfan sulfate, 16. p,p'-DDT, 17. Endrin ketone, 18. Methoxychlor. Peak # 17 (Endrin ketone) is a degradation product of Peak # 11 (Endrin).

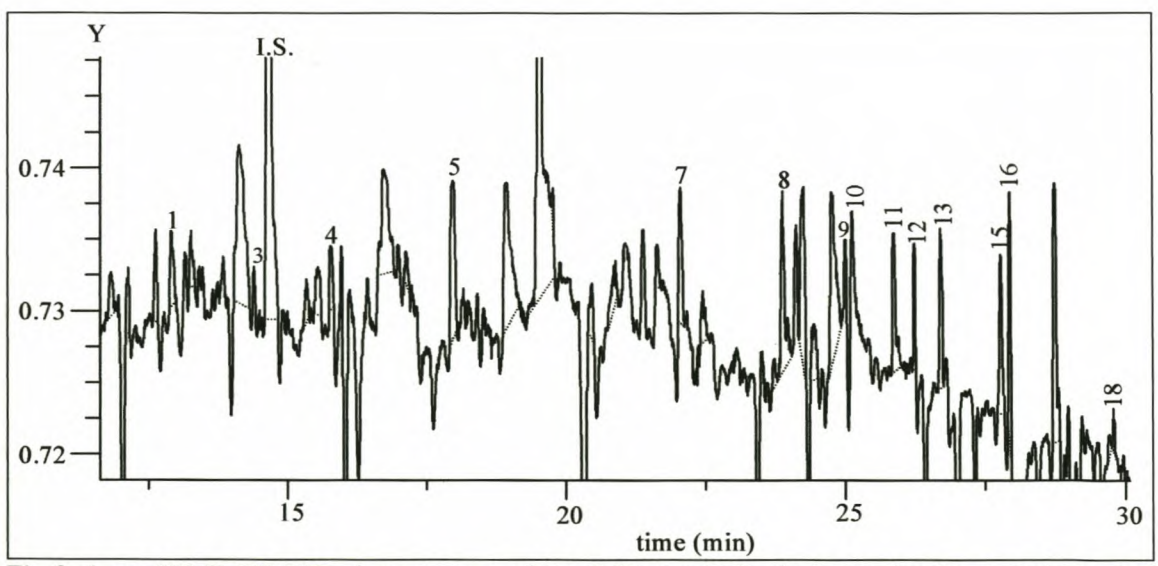


Fig 2. 1 ppt SPME-GC-ECD chromatogram of 17- OCP standards obtained using N_2 as make-up gas. Except Peaks # 2, 6,14, and, 17 all can be clearly seen.
NB: For compound identification see Fig 1.

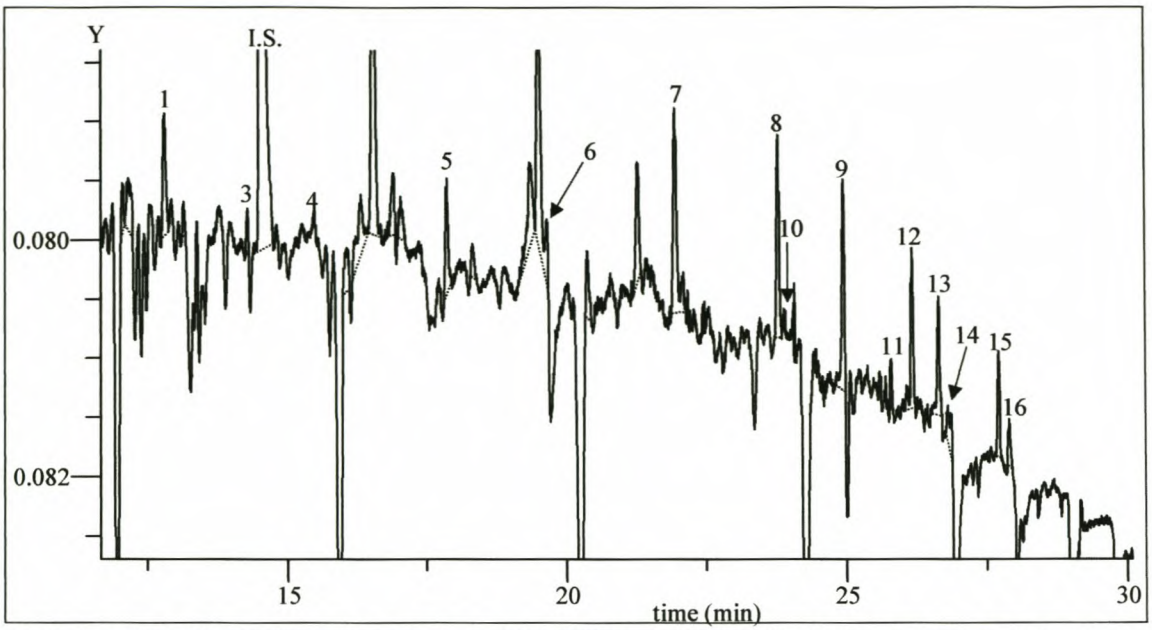


Fig 3. 1 ppt SPME-GC-ECD chromatogram of 17- OCP standards obtained using Ar/CH_4 as make-up gas. Except Peaks # 2, 17 and 18 all can be clearly seen.
NB: For compound identification see Fig 1.

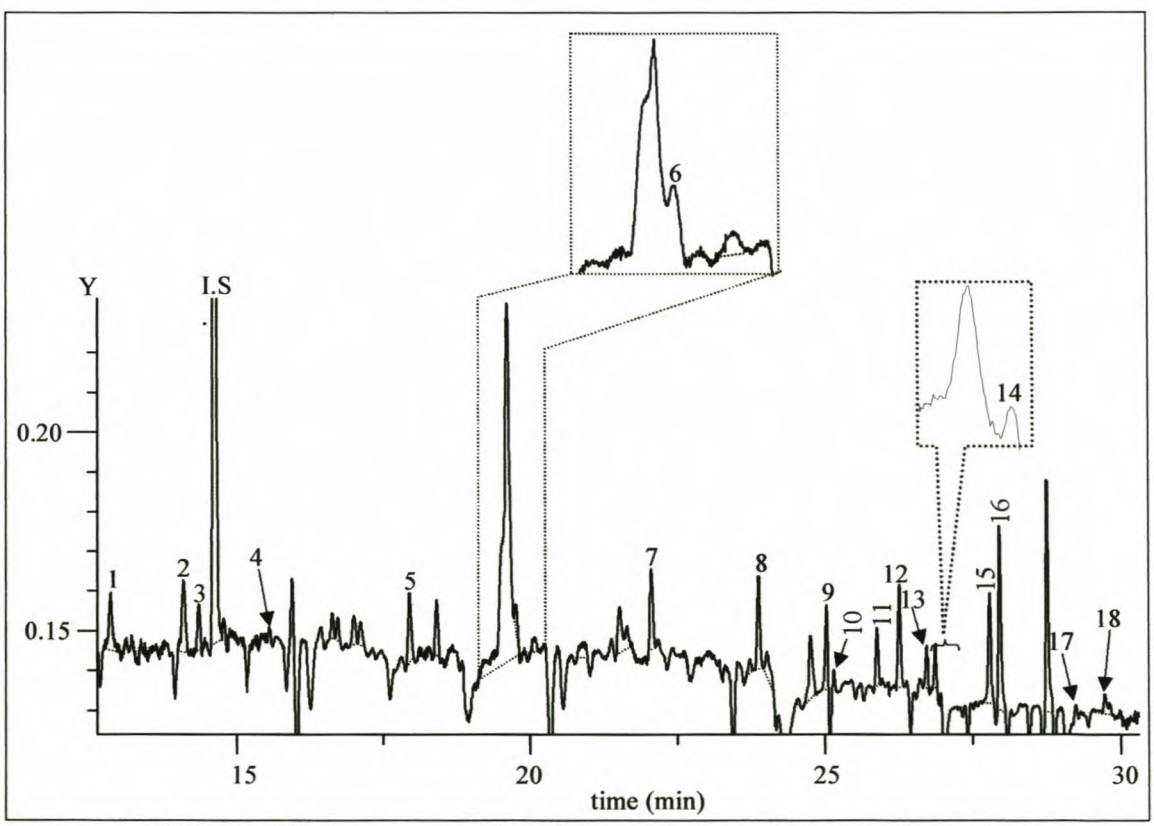


Fig 4. 5 ppt SPME-GC-ECD chromatogram of 17- OCPs standards obtained using N_2 as make-up gas.
NB: For compound identification see Fig 1.

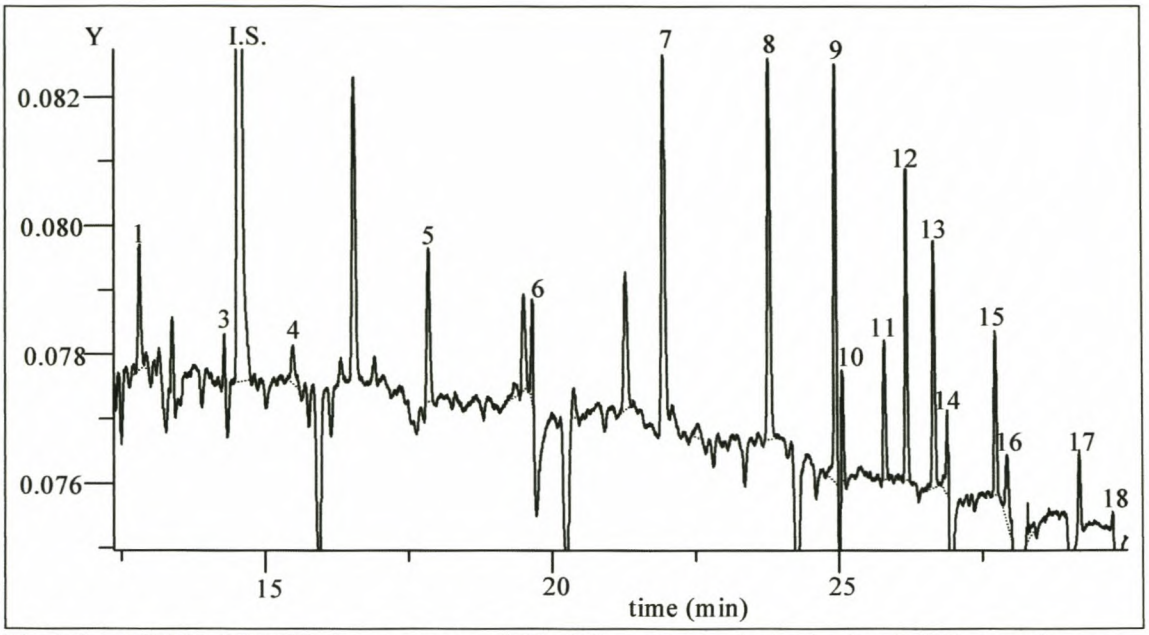


Fig 5. 5 ppt SPME-GC-ECD chromatogram of 17- OCP standards obtained using Ar/CH_4 as make-up gas. Except Peaks # 2 all can be clearly seen.
NB: For compound identification see Fig 1.

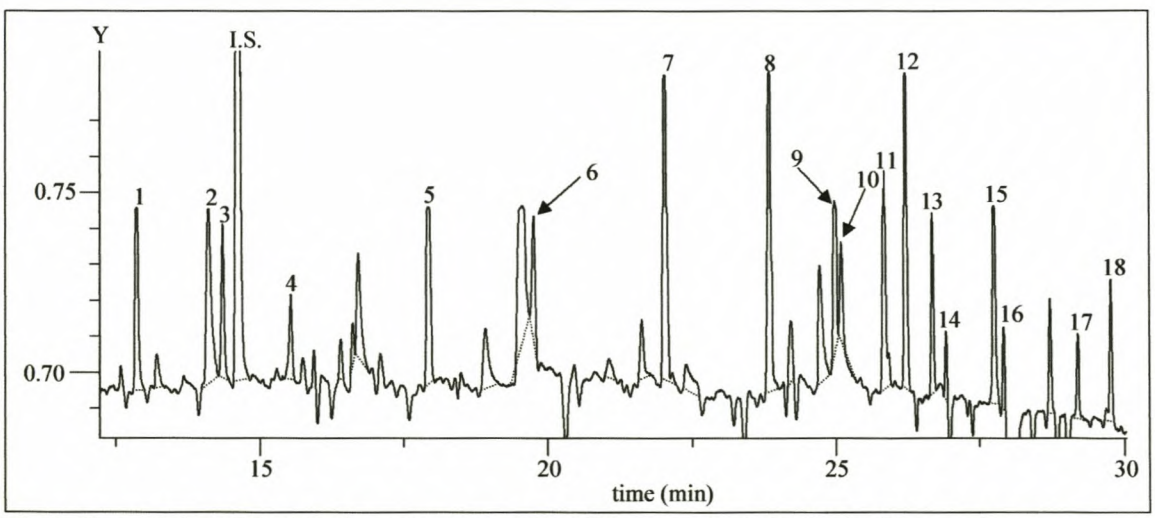


Fig 6. 50 ppt SPME-GC-ECD chromatogram of 17- OCP standards attained using N_2 as make-up gas.
NB: For compound identification see Fig 1.

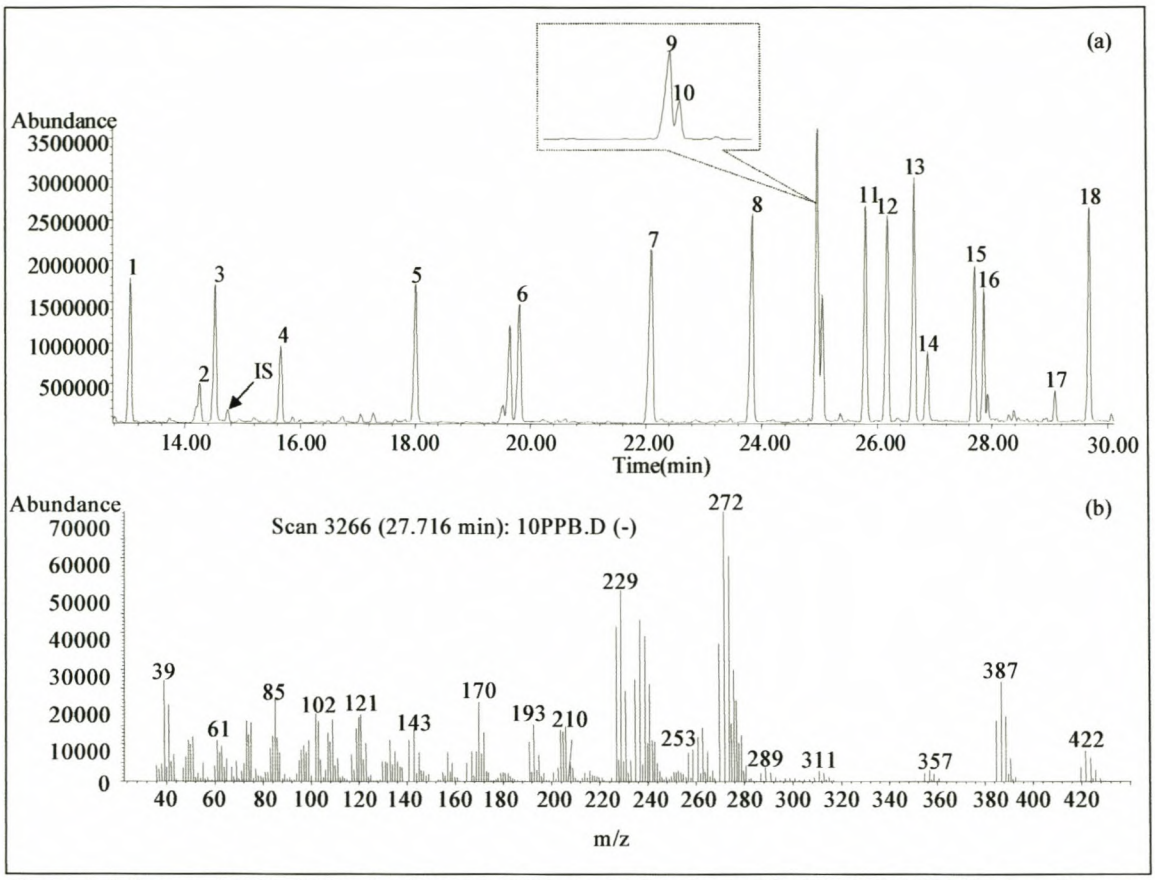


Fig 7. A full scan a) TIC of 10 ppb of the 17- OCP standards using TDS-PTV-GC-MSD b) Mass Spectrum of endosulfan sulfate.
NB: For compound identification see Fig 1.

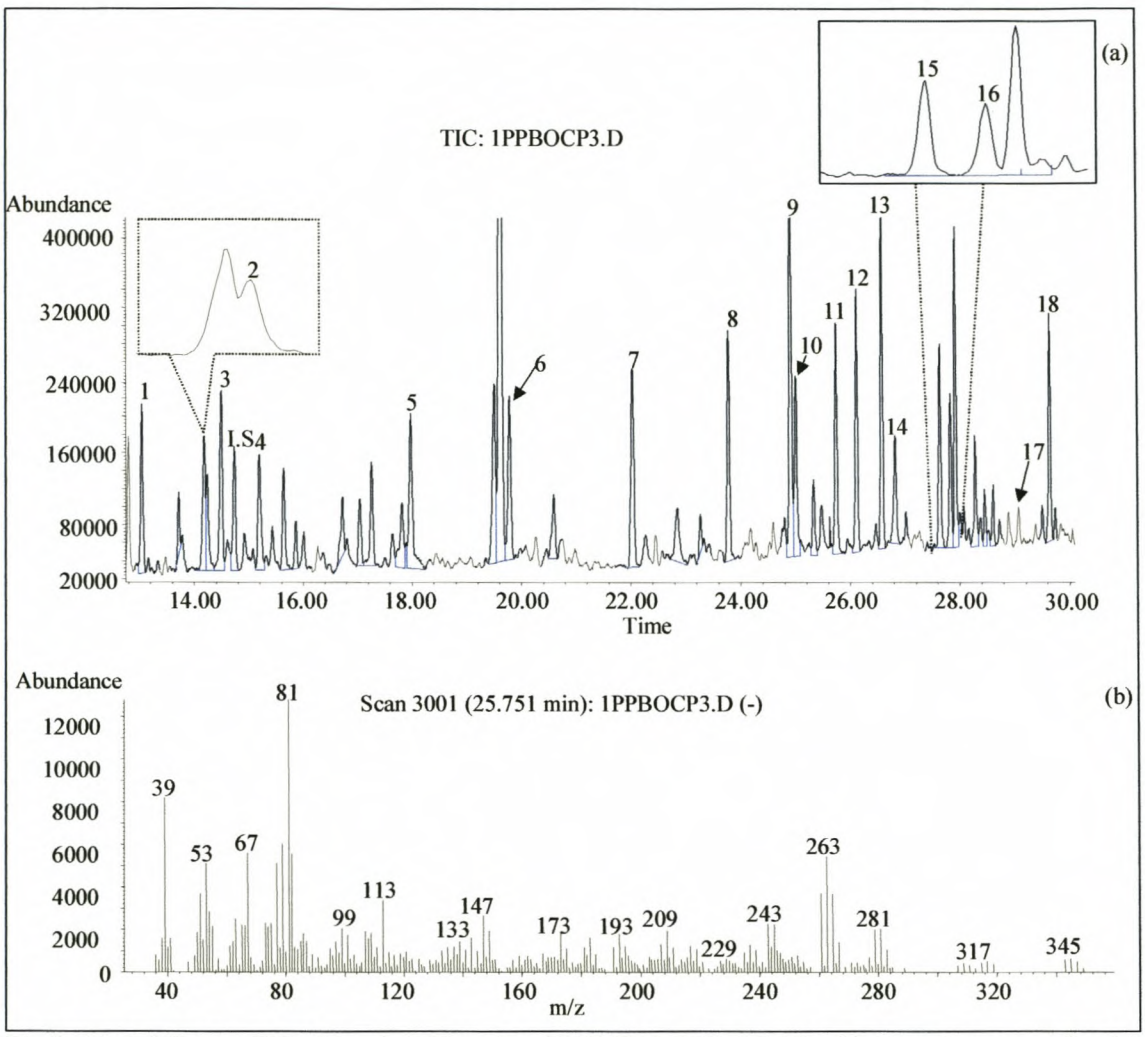


Fig 8. (a) A full scan TIC of 1 ppb OCPs using SBSE-TDS-PTV-GC-MS, (b) Mass Spectrum of endrin. **NB**: For compound identification see Fig 1.

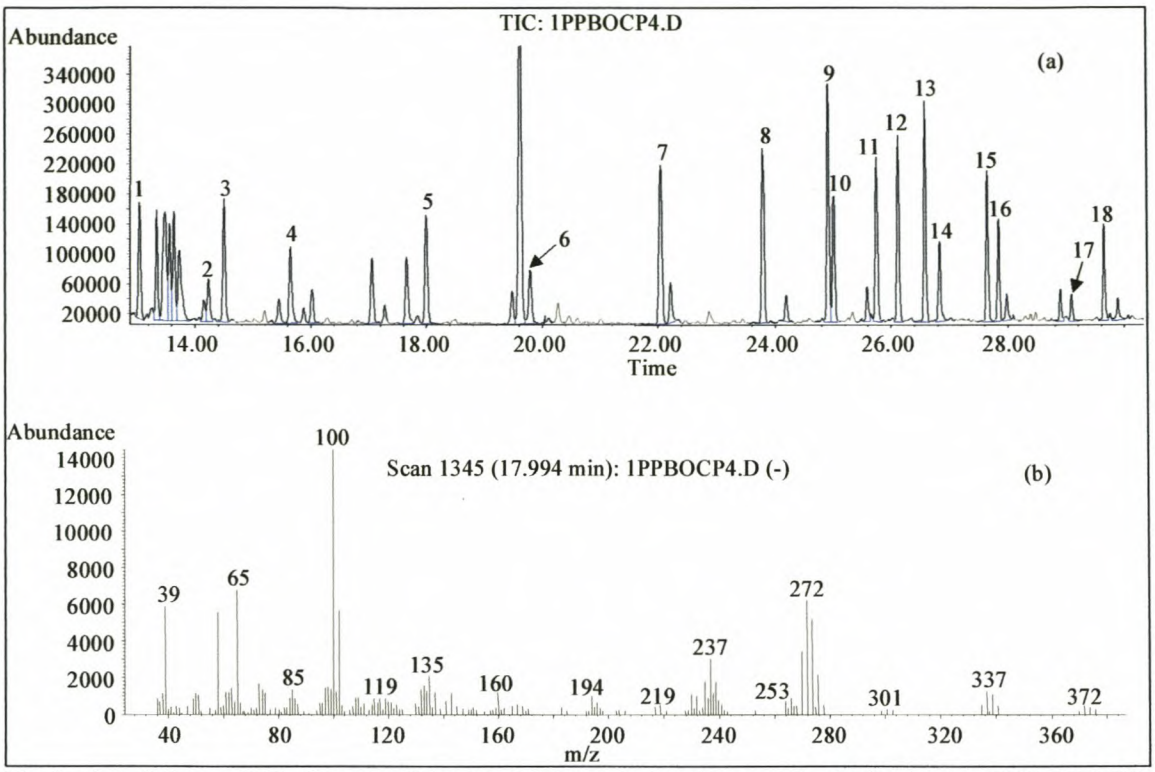


Fig 9. a) A full scan TIC of 1 ppb of the 17- OCP standards with out I.S. obtained using TDS-PTV-GC-MSD b) Mass Spectrum of heptachlor.
NB: For compound identification see Fig 1.

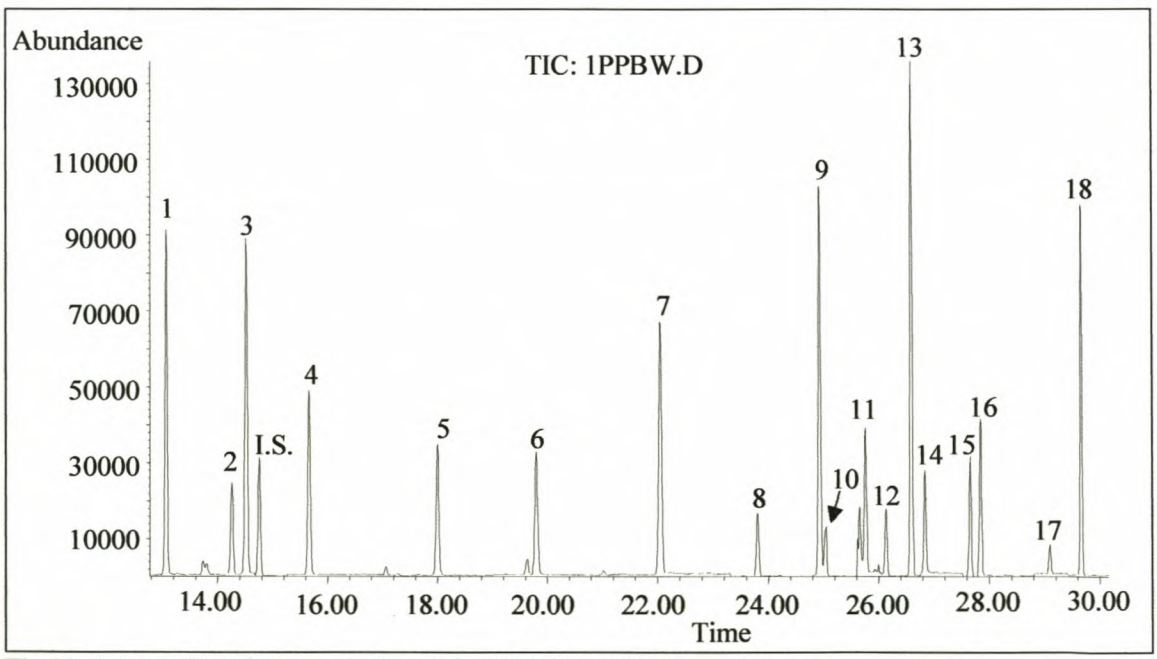


Fig 10. A SIM of 1 ppb OCPs obtained using SBSE-TDS-PTV-GC-MS.
NB: For compound identification see Fig 1.

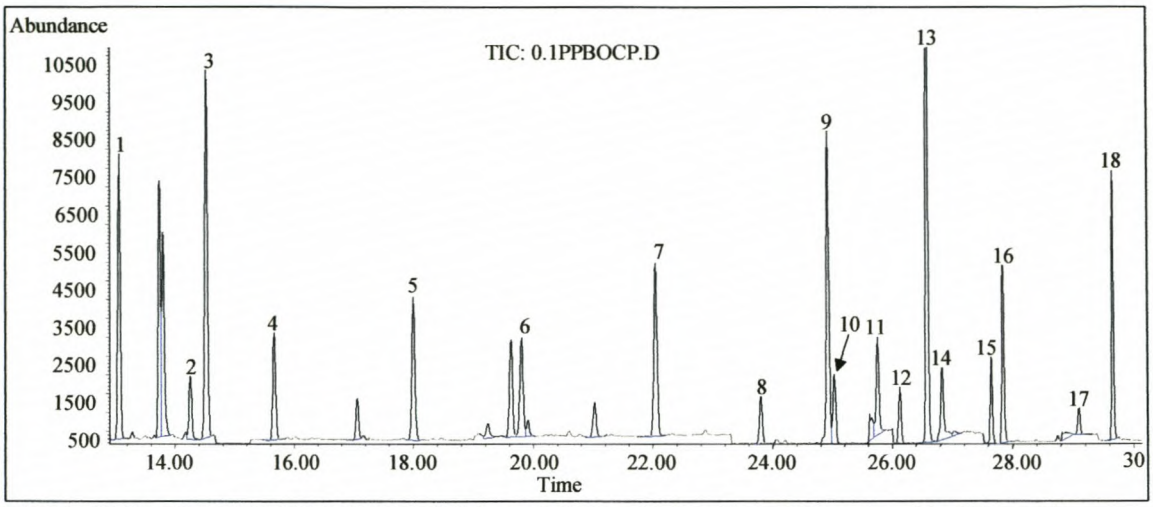


Fig 11. SBSE-TDS-PTV-GC-MS TIC of 100 ppt OCP standards without I.S. analysed in SIM mode.
NB: For compound identification see Fig 1.

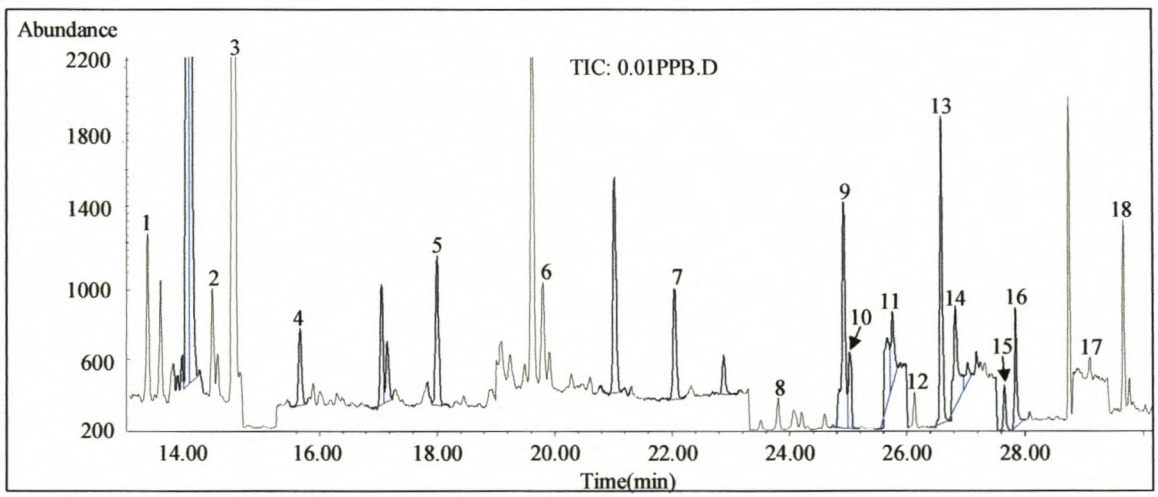


Fig 12. SBSE-TDS-PTV-GC-MS TIC of 10 ppt OCP standards without I.S. performed in SIM mode.
NB: For compound identification see Fig 1.

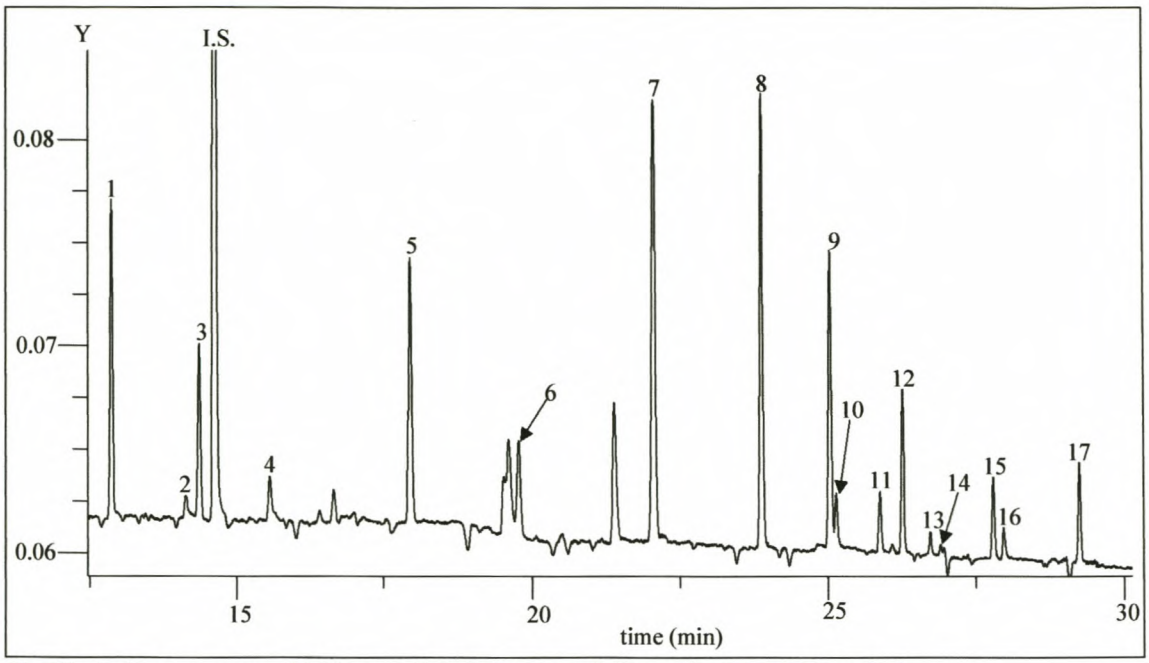


Fig 13. A chromatogram of 100 ppt soil-SPME extract followed by GC-ECD analysis.
NB: For compound identification see Fig 1.

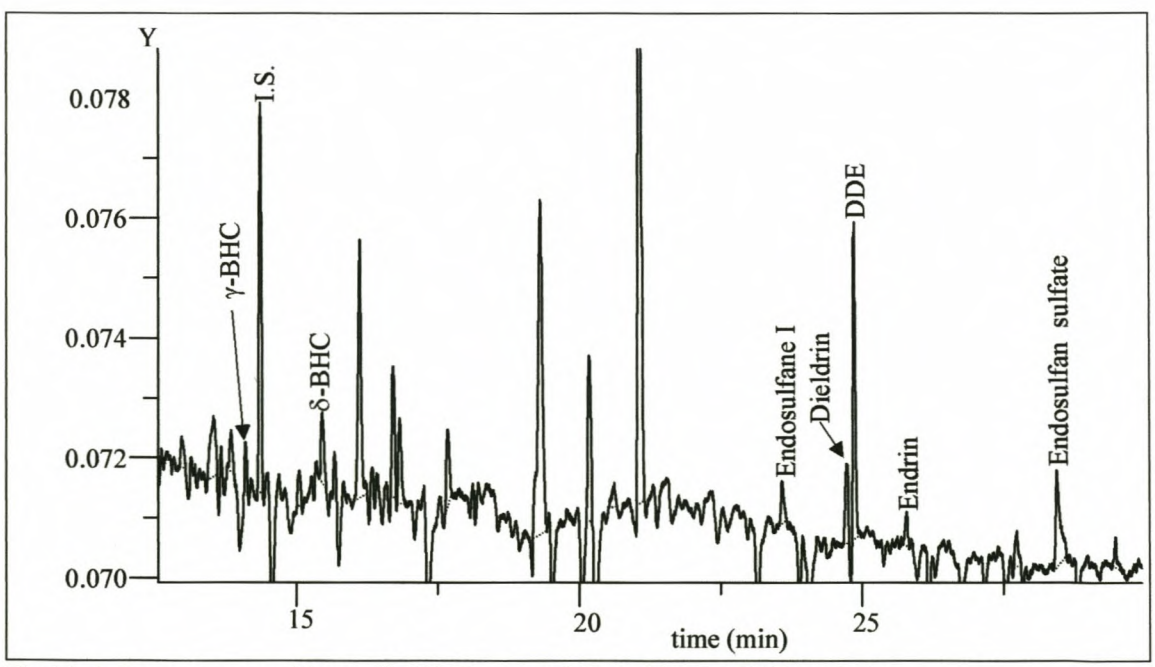


Fig 14. A chromatogram of 1ppt soil-SPME extract followed by GC-ECD analysis.

Appendix B

SPME-GC-ECD Chromatograms of water samples collected from Stellenbosch and Eritrea.

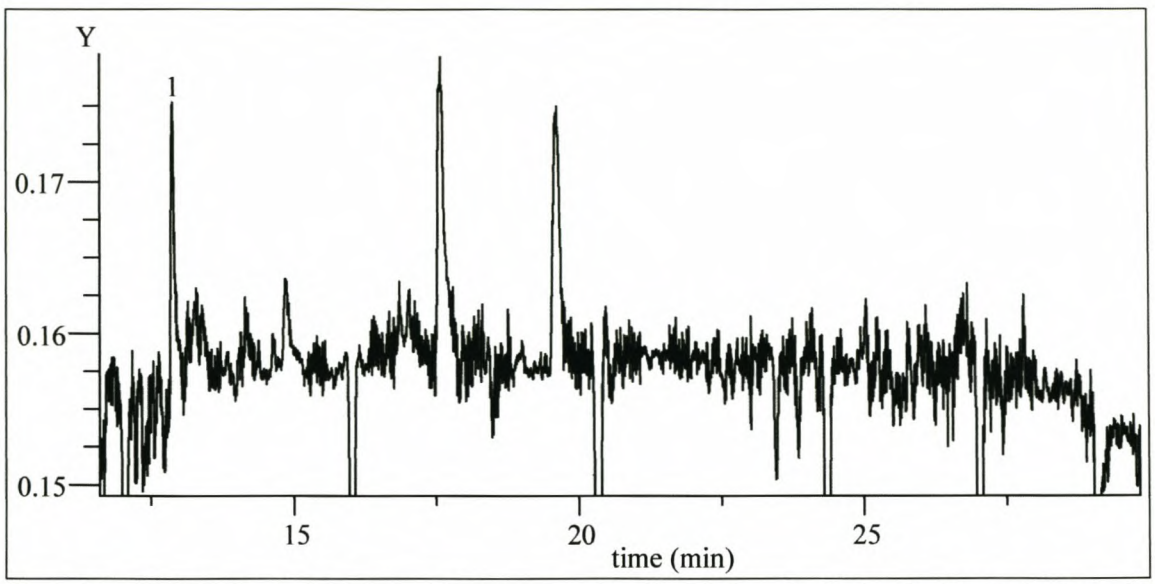


Fig 1. SPME-GC-ECD chromatogram of a water sample collected from Stellenbosch close to the Parmalat milk factory. 64 ppt. α-BHC was detected (1). Quantification was done by external calibration.

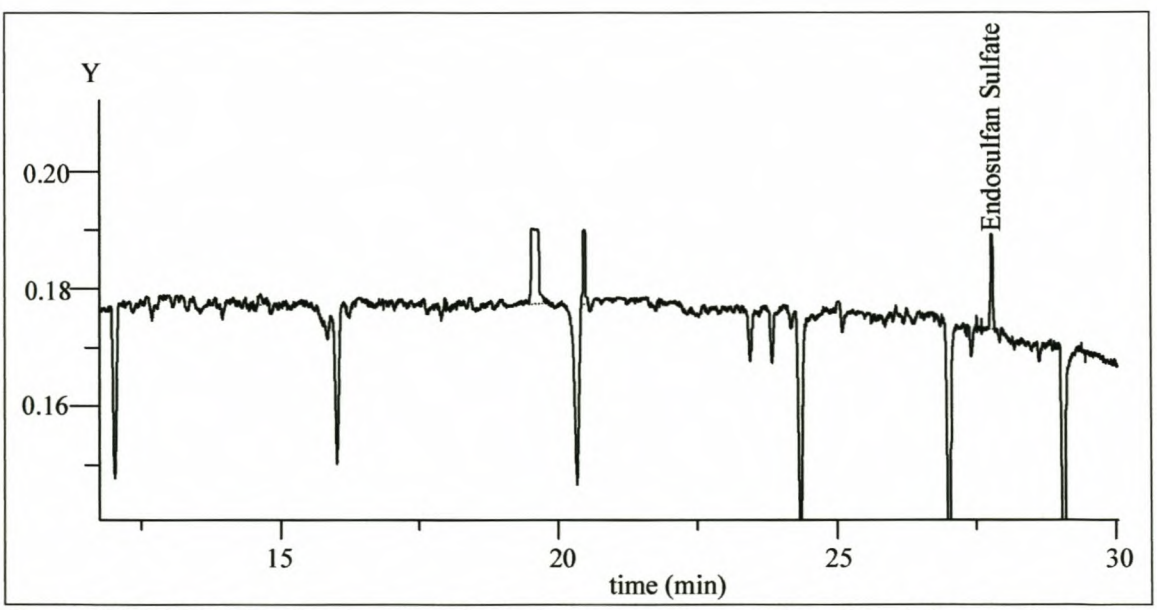


Fig 2. SPME-GC-ECD chromatogram obtained from a water sample collected from a ditch in the Paradijskloof area of Stellenbosch. 75 ppt endosulfan sulfate was detected. Quantification was done by external calibration.

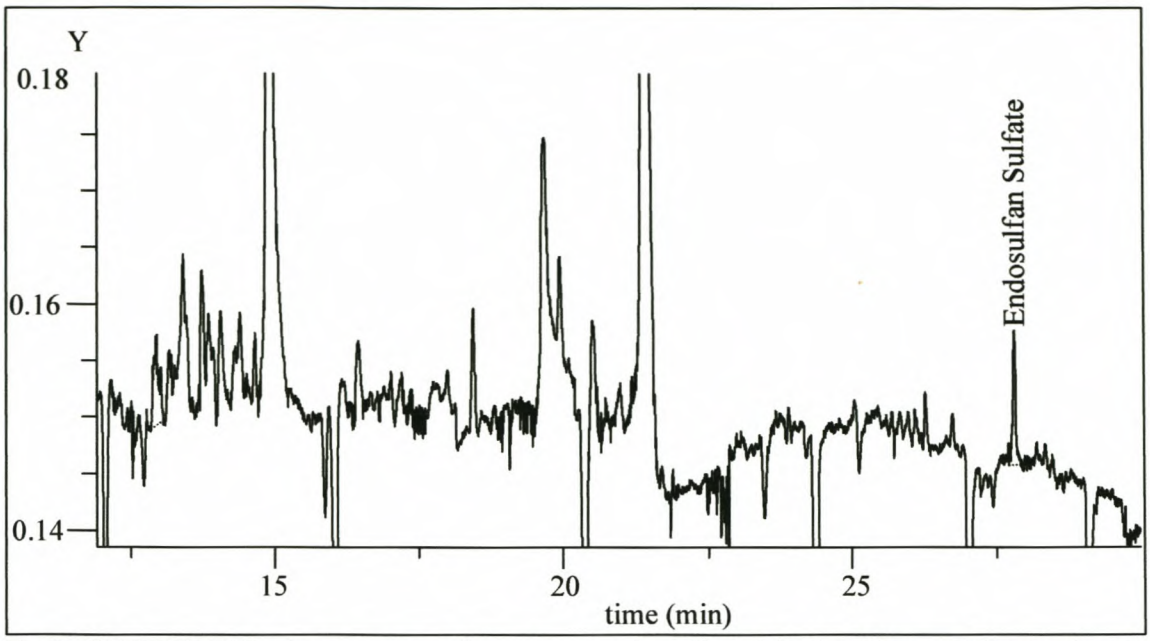


Fig 3. SPME-GC-ECD chromatogram of a water sample collected in the Johannesdal area close to Sellenbosch. Endosulfan sulfate was detected (72ppt). Quantification was done by external calibration.

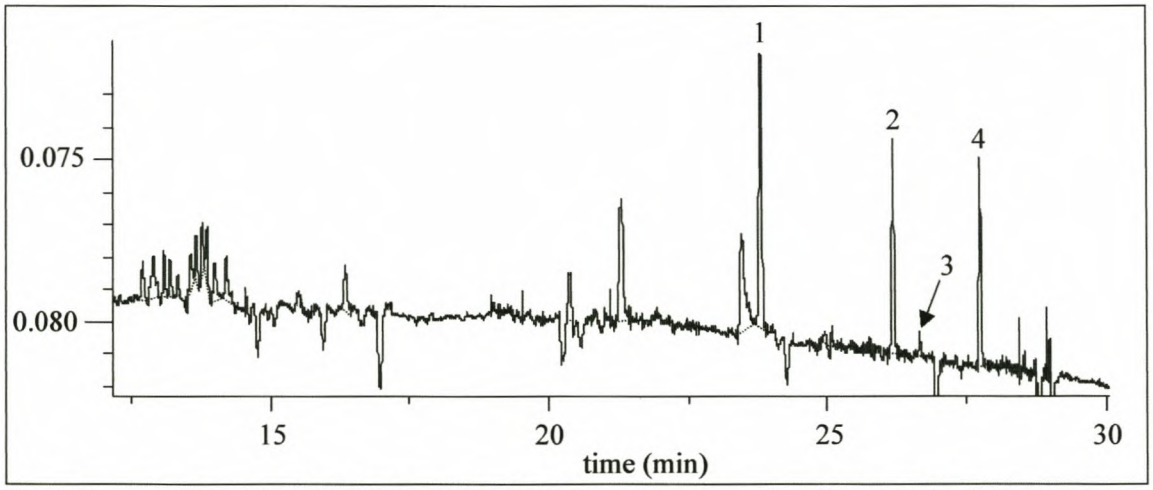


Fig 4. Chromatogram of an SPME-GC-ECD extract from a water sample collected in the Stellenbosch area close to the Tokara farm. The detected pesticides are: 1) endosulfan I (203 ppt), 2) endosulfan II (120 ppt), 3) p,p'-DDD (15 ppt) 4) endosulfan sulfate (123 ppt). Quantification was done by external calibration.

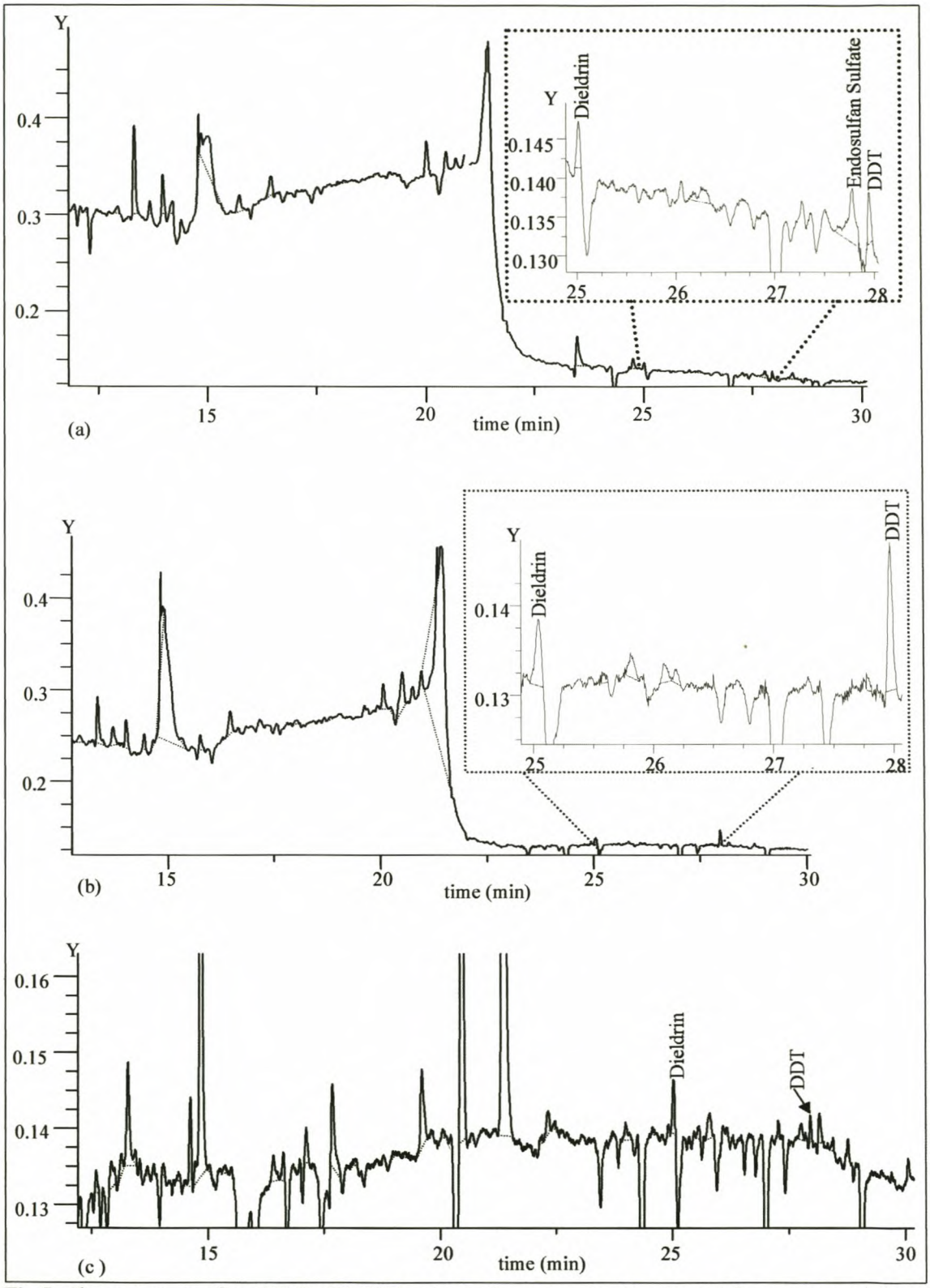


Fig 5. Chromatogram of an SPME-GC-ECD extract of a water sample collected from a reservoir in the Devan's valley area close to Stellenbosch a) without any matrix adjustment b) after adjusting the pH of the sample to 9.7 c) after adjusting the pH of the sample to 11.9.

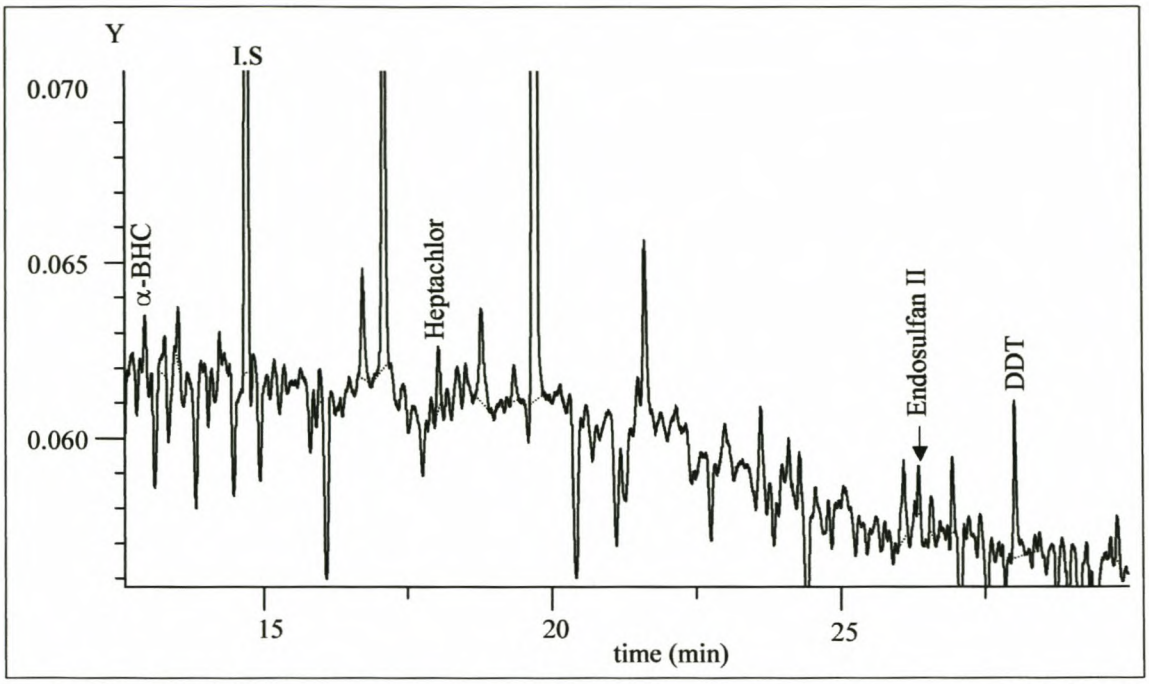


Fig 6. SPME-GC-ECD chromatogram of sample EWS9. The detected pesticides were α-BHC (9 ppt), heptachlor (15 ppt), endosulfan II (5 ppt), p,p'-DDT (112 ppt). Quantification was done by standard addition (internal calibration).

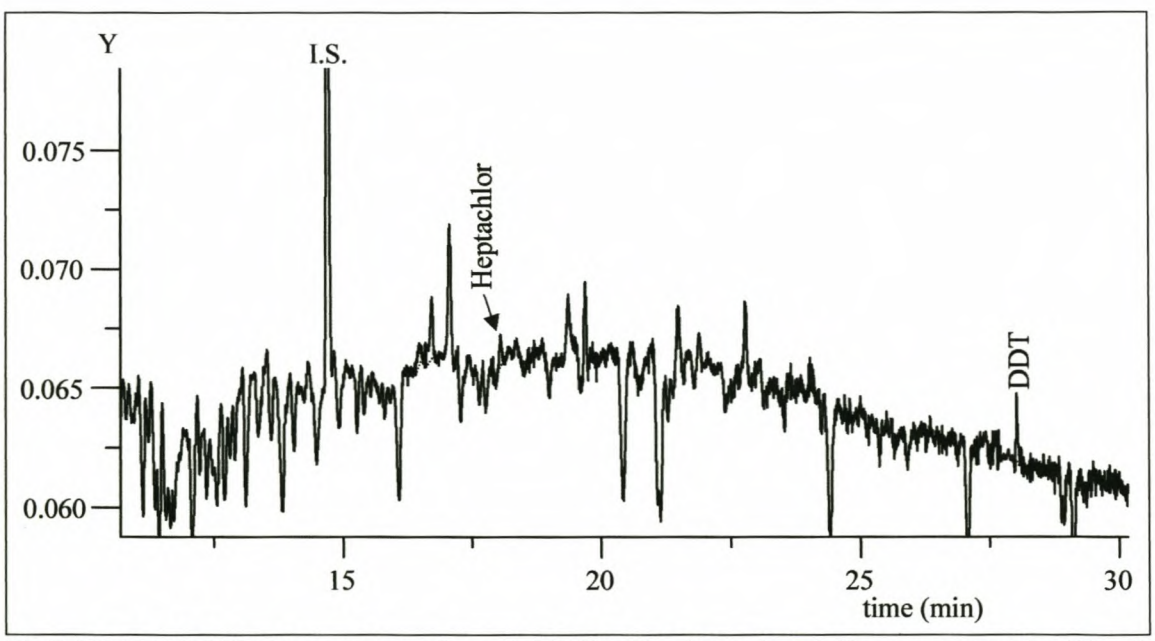


Fig 7. SPME-GC-ECD chromatogram of sample EWS12. The detected pesticides were heptachlor (10 ppt), p,p'-DDT (41 ppt). Quantification was done by standard addition (internal calibration).

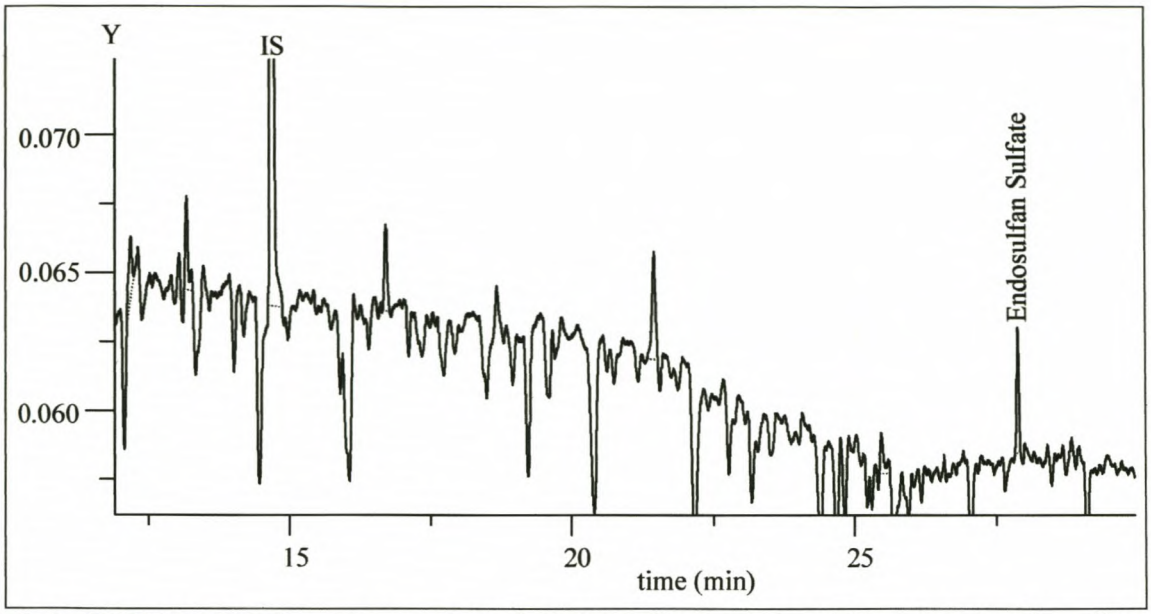


Fig 8. SPME-GC-ECD chromatogram of sample EWS17. The detected pesticide was endosulfan sulfate-(31 ppt). Quantification was done by standard addition (internal calibration).

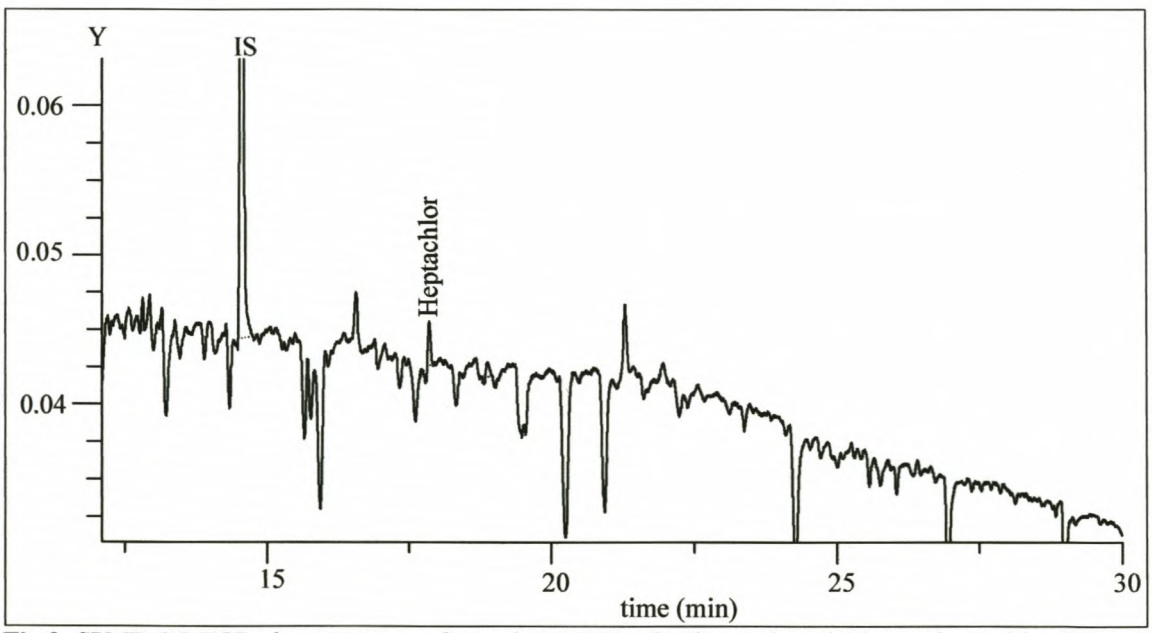


Fig 9. SPME-GC-ECD chromatogram of sample EWS20. The detected pesticide was heptachlor (14 ppt). Quantification was done by standard addition (internal calibration).

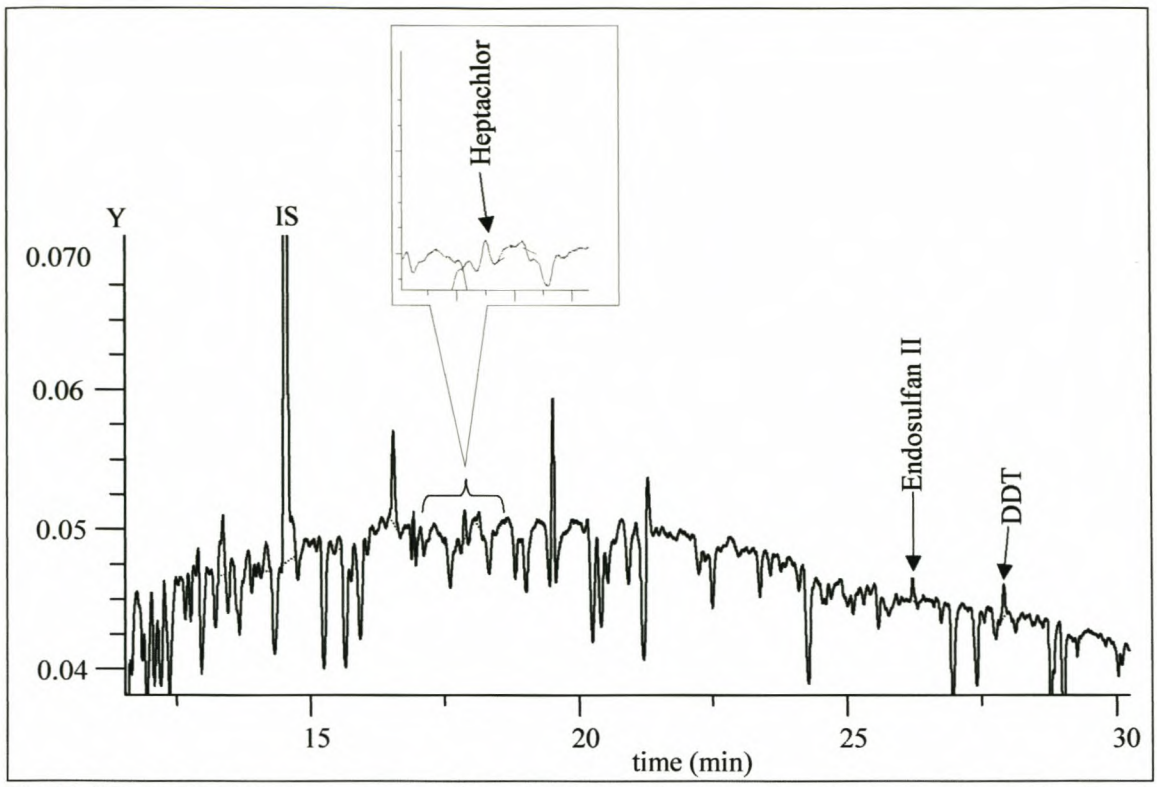


Fig 10. SPME-GC-ECD chromatogram of sample EWS21. The detected pesticides were heptachlor (6 ppt), endosulfan II (3 ppt), p,p'-DDT (24 ppt). Quantification was done by standard addition (internal-calibration).

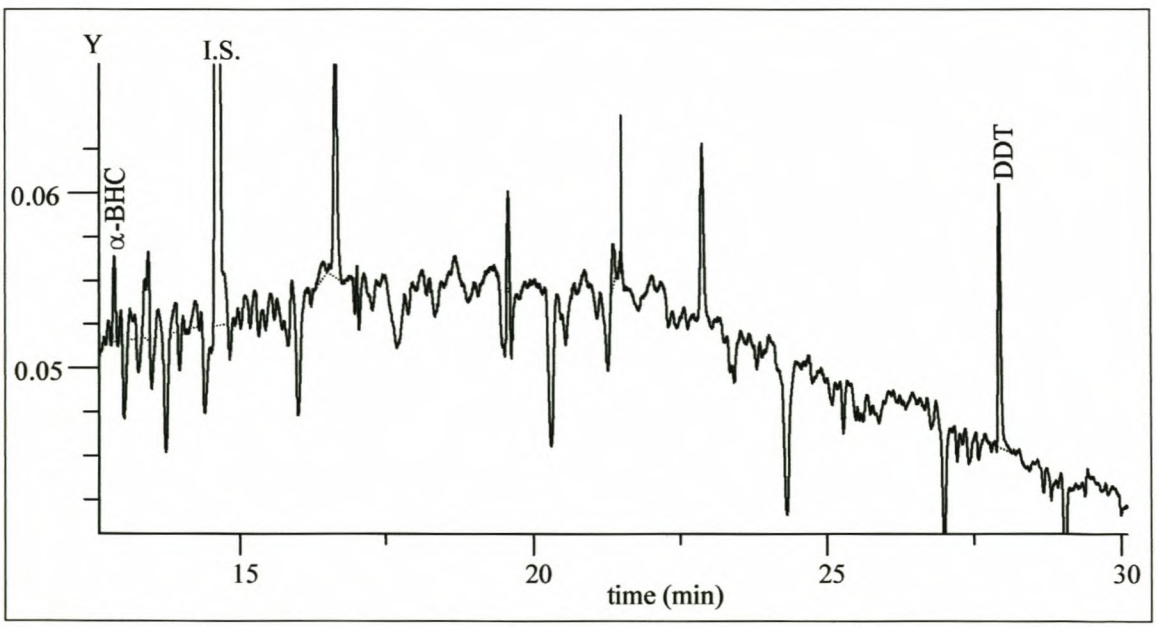


Fig 11. SPME-GC-ECD chromatogram of sample EWS24. The detected pesticides were α-BHC (7 ppt), p,p'-DDT (67 ppt). Quantification was done by standard addition (internal calibration).