COST EFFECTIVE METHODS FOR MONITORING PESTICIDE POLLUTION IN WATER SYSTEMS

Report to the
WATER RESEARCH COMMISSION

by

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WRC Report No 1120/1/04
ISBN No 1-77005-117-1

MARCH 2004

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ACKNOWLEDGEMENTS

The research in this report emanated from a project funded by the Water Research Commission entitled:

"Cost effective methods for monitoring pesticide pollution in water systems: technologies and procedures for field use in rural areas [Project Number K5/1120/0/1]"

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The financing of the project by the Water Research Commission and the contribution of the members of the steering committee is gratefully acknowledged. The project was only possible with the co-operation of many individuals and institutions. The authors therefore wish to record their sincere thanks to the following:

Dr E Poole - University of Stellenbosch
Mr S Hassan - Peninsula Technikon
Mr B Delcarme - Peninsula Technikon
Miss E Sinanovic- University of Cape Town
The environmental health students from Peninsula Technikon who conducted the health survey
The Hexriver Producers Association
All the environmental health officers, farmers and farm workers who participated in the health survey

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DEFINITION OF ACRONYMS

ADI	Accepted Daily Intake
ARC	Agricultural Research Council
CSIR	Council for Scientific and Industrial Research
DWAF	Department of Water Affairs
EHO	Environmental Health Officer
ELISA	Enzyme-linked Immunoassays
EPA	Environmental Protection Agency
EU	European Union
HPLC	High Pressure Liquid Chromatography
GC-Gas	Gas Chromatography
IM-AS	24 hour autosampling SPME
IM-TWA	24 hour TWA SPME
IPM	Integrated Pest Management
MDL	Method Detection Limit
NGO	Non Governmental Organisation
PDMS	Polydimethyl siloxane
SABS	South African Bureau of Standards
SAIMR	State Laboratory in Woodstock, Cape Town
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
TWA	Time Weighted Average
WHO	World Health Organisation
WWF	World Wild Life Fund

DEFINITION OF SYMBOLS

A	Cross-sectional area
C	Concentration
C_f	Concentration of analyte in the fibre coating
C_f*	Dimensionless concentrations in the fibre
C_{Fcl}	The fibre concentration at the slab (model) centreline corresponding to the end of the fibre
C_{fi}	The concentration at the interface between the fibre and the sample, on the fibre side
C_i	Concentration during at time interval i
C_s	Concentration of analyte in the sample
$C_{s¥}$	Sample concentration at 'infinity', a distance sufficiently far from the sample to be unaffected by mass transfer of the analyte to the fibre (free stream concentration)
C_{s0}	The initial concentration of the fibre and the sample in the vicinity of the fibre (C_{s0} = 0)
C_{TWA}	Time Weighted Average Concentration
D_{AB}	The diffusivity of substance B through A
δ_χ	The concentration boundary layer thickness
δ_ϕ	Film thickness (on the sample side)
D_f	Diffusivity of the analyte in the fibre
D_s	Diffusivity of the analyte in the sample
$\Delta\xi$	Incremental distance
$\Delta\xi$	Retracted a distance of the fibre into the steel shaft
ϕ_B	The association parameter
j	The mass flux, with units of mass/(time*area)
kc	Mass transfer coefficient
K_{fs}	The distribution constant (partition coefficient)
l_f	The fibre length
m	Mass (of analyte)
M_B	The molecular mass of solvent B
μ_B	The viscosity of B
m_f	Mass of analyte absorbed into the fibre coating
MT	Mass Transfer
m_T	The total mass absorbed onto the fibre, to time t_T
R	The volumetric sampling rate, with units of volume/time.
Re	Reynold's Number
Sc	Schmidt Number
t	Time
T	The absolute temperature
t_f*	Dimensionless time, based on fibre diffusivity
t_i	The ith time interval
t_s*	Dimensionless time, based on sample diffusivity
t_T	Total sampling time
$u_\square$	Velocity at 'infinity'
µg	microgramme
V_A	The molal volume of solute A at the normal boiling point
V_f	Fibre coating volume
x	Distance
x*	Dimensionless distance
x_1	Thickness of the (infinite) slab, length of the fibre

Executive Summary

BACKGROUND

South Africa is the largest market for pesticides in sub-Saharan Africa. Despite this, relatively little is known about the environmental and health impacts of widespread pesticide use, particularly in rural agricultural areas. Previous research supported by the Water Research Commission (WRC Report No. 795/1/00) found consistent low level pesticide contamination of rural water sources in three Western Cape agricultural areas and highlighted the need for monitoring of water sources for pesticides. In response to this finding, a 3-year follow-up study between 2000 and 2002 was undertaken to explore the potential development of cost-effective methods for monitoring for pesticide pollution. The research was conducted collaboratively by the Occupational and Environmental Health Research Unit at the University of Cape Town and the Departments of Analytical Chemistry and Chemical Engineering at the Peninsula Technikon (PENTECH) - the same group that completed the baseline contamination study from 1997 to 1999.

The study was motivated by the fact that in South Africa, infrastructure is poorly developed to monitor and control pesticides reaching water sources, particularly in rural farming areas. Methods for the detection of pesticides in water are currently constrained by a number of practical limitations: 1) relatively few laboratories undertake pesticide analysis and few provide details of rigorous quality assurance; 2) high expense of testing; 3) barriers posed by commercialisation of testing services, and 4) practical difficulties relating to collection and transport of samples. All these factors result in poor availability of testing.

Moreover the human resource and technical, support available in rural communities to implement any testing programmes for pesticides is largely unknown.

The project therefore addressed some of these serious gaps in the environmental management of pesticides. At the same time, the project intended to contribute both to improving quality of rural life, while at the same time promoting environmental justice and empowerment of rural communities. It is these vulnerable groups, rural communities and farm workers, who are most at risk from undetected and uncontrolled pesticide exposures in water.

AIMS AND OBJECTIVES

The aim of the project was to evaluate cost-effective methods for monitoring pesticide pollution in rural water.

The objectives were:

1. To evaluate the utility of solid-phase micro-extraction (SPME) fibres and a programmable sampler as tools to achieve integrated sampling of water sources being tested for pesticide pollution.
2. To evaluate the utility of enzyme immunoassay kits for the quantitative and semi-quantitative identification of pesticides in water .
3. To develop a set of procedures /guidelines for use by rural communities and/or rural local authorities for monitoring water sources for pesticide pollution (based on the

results of objectives 1 and 2, in conjunction with findings of the WRC project No. 795/1/00).

4. To estimate the costs associated with the procedures evaluated under objectives 1 and 2, and compare these costs to conventional methods for monitoring.

5. To formulate the procedures evaluated under objectives 1 and 2 in the form of a manual.

6. To evaluate the capacity existing in rural communities and local authorities to implement monitoring of water sources for pesticides, and to make recommendations for strengthening such capacity.

The project therefore comprised a set of discrete sub-studies, which included *in-situ* laboratory development (SPME utility), analysis of field samples (SPME utlility, immunoassay testing), field surveys (human resource and community capacity) and health economic analysis (costing). The study area and sample sites were those that had been extensively studied by the research team in the previous investigation. The Hex River district, an intensive grape-farming area with high pesticide usage was therefore chosen as the study area for this project. Samples were taken from 2 points along the course of the Hex River which represented a sequential flow downstream, two water reservoirs situated alongside vineyards, and a drain in the vineyards that collected water from soil surface run-off. Similarly, the two pesticides examined, endosulfan and chlorpyrifos, were those previously found to be most prevalent in the region.

THE UTILITY OF SOLID-PHASE MICRO-EXTRACTION (SPME) FIBRES FOR MEASURING PESTICIDE CONTAMINATION IN WATER

Solid-phase microextraction (SPME) utilizes a short, thin, solid rod of fused silica that is coated with an absorbent polymer. The coated SPME fibre is attached to a metal rod, and both are protected by a metal sheath that covers the fibre when not in use. Solid phase microextraction (SPME) was introduced to eliminate the problems associated with Solid Phase Extraction (SPE). SPME sampling involves the extraction of an analyte into the polymer coating, an equilibrium process based on the partitioning of the analyte between the sample phase and the polymeric coating. By using SPME rather than SPE, solvents are completely eliminated, blanks are greatly reduced and extraction times may also be greatly reduced. It is not only a quicker alternative, but potentially a more cost-effective one as well.

The experimental work confirmed that vigorous stirring (agitation) of the sample is essential in order to achieve reproducible results. A series of experiments using absorption times of 5 to 25 minutes, in increments of 5 minutes, showed that the improvement in reproducibility beyond an absorption time of 20 minutes for the two pesticides under consideration, was neglibile. An absorption time of 25 minutes was used in the majority of subsequent analyses as this conveniently coincides with Gas Chromatograph (GC) cycle times, and is similar to absorption times reported in the literature.

The time required to achieve equilibrium (maximum) absorption was explored by increasing the absorption time from 20 minutes to 140 minutes, at intervals of 20 minutes. Maximum absorption, for the analytes concerned and under the experimental conditions, occurred for an absorption time between 60 and 80 minutes.

The reproducibility of the SPME/GC sampling and analytical method was assessed by comparing the differences between replicate pairs. Under the optimum agitation conditions (N = 625rpm) and the absorption time (25 minutes) established for the analytes under consideration, the SPME method yielded

excellent reproducibility. The relative standard deviation of the differences between 17 replicate standard analyses was about 1% for 3 of the analytes, and less than 10% for endosulfan II, for standards with concentrations in the range 0.1 to 5µg/L. For the 3 field samples analysed, the relative differences between replicates ranged between 1 and 10% for the 4 analytes under consideration, for analyte concentrations in the range 0.1 to 6.0 µg/L.

Method Detection Limits were estimated based on a ratio of 3x the Standard Deviation of a set of 7 determinations, similar to methods recommended by the US Environmental Protection Agency (USEPA). Limits of 0.01µg/L to 0.02 µg/L were established for the analytes of interest.

The SPME method is quicker and simpler to use than traditional Solid-Phase Extraction. The fibres are reusable for up to 100 analyses. When compared to other techniques of analysis, such as Solid Phase Extraction (SPE) and Enzyme Linked Immunoassays (ELISA), the SPME method rendered more reliable results on a consistent basis without being time consuming or labour intensive. Both reproducibility and detection limits are superior to the SPE method.

THE UTILITY OF SOLID-PHASE MICRO-EXTRACTION (SPME) FIBRES AND A PROGRAMMABLE SAMPLER AS TOOLS TO ACHIEVE INTEGRATED (TIME WEIGHTED AVERAGE) SAMPLING OF WATER SOURCES BEING TESTED FOR PESTICIDE POLLUTION

An accurate assessment of the water quality in a particular region currently requires a relatively frequent (and costly) sampling regime because it relies on multiple grab samples to characterise contamination over a particular period. However, the costs of attempting to assess water quality at a number of sites through frequent grab sampling are prohibitive. In practice, grab samples are taken at intervals varying from twice per week to weekly or at greater time intervals, and average values are inferred from what are actually relatively infrequent grab samples. A sampling method that yields a Time Weighted Average (TWA) concentration over a comparatively extended period (24 hours or longer), using a single sample, would give a more accurate picture of prevailing contaminant levels.

An unsteady state theoretical analysis of the rate of mass transfer of the analyte (pesticide) from the sample, assumed to be flowing perpendicular to the face of the fibre, to the fibre is presented. The theoretical analysis develops previous steady state analyses, used to establish the basis for measuring TWA concentration of volatile or semi-volatile compounds in air, and uses the mathematical results of analogous unsteady state heat transfer problems. The theoretical analysis presented in this report showed that the SPME system could be adapted to obtain a TWA sample of pesticides in water, at concentrations of 1 µg/L. The system could be operated with mass loadings of 30-40% of the equilibrium values, with a corresponding increase in method sensitivity (detection limit) provided that the linearity of the mass loading rate through the sampling period was preserved by operating the system at a sufficiently low Mass Transfer Biot number for the sample period under consideration. The theoretical analysis also demonstrated the impossibility of satisfying both linearity and sensitivity criteria with a single fibre located in a particular configuration if the analytes of interest have a wide range of distribution constants (K_{fs} values) – in this case the ratio of the highest to lowest K_{fs} values, based on literature data, is approximately 50:1. A multi-fibre system is required for these cases. Temperature dependence of K_{fs} values may be significant, therefore in-field temperature control of the fibre should be considered for maximum reproducibility.

In-situ laboratory experiments were conducted to estimate: 1) the diffusivities in water of the two analytes under

investigation 2) the distribution constant values (K_{fs}) 3) analyte retention on the fibre 4) linearity of mass absorption with respect to time and concentration, and 5) the effect of exposure concentration profiles on detections. Comparison was then made between the modelled TWA based on SME analysis and average concentration of 24 separate hourly grab samples collected by an autosampler.

The experimental results confirmed that in principle and in a practical environmental setting, the SPME device could be adapted to obtain a TWA sample of the pesticides of interest, over a sampling period of up to 3 hours. The laboratory-based experiment showed that a similar (agreement within 6%) TWA sample was obtained for two markedly different exposure patterns with the same theoretical TWA concentration, a key requirement for the success of the method.

The field test over 24 hours showed that a TWA sample could be obtained with minimal effort. However, the TWA sample result significantly underestimated the average concentrations of 24-hourly samples. However, the fibre failed to retain, for the time period required, 100% of the analyte absorbed, with losses of over 50% over a 2-hour period. Based on limited experimental data, this loss factor was assumed to be constant, and the calculated TWA result was adjusted accordingly. The validity of this calculation procedure has yet to be confirmed experimentally. The loss of analyte clearly degrades the sensitivity (and possibly the reproducibility as well) of the method. The losses over 24 hours may be as high as 90%. The loss of analyte from the fibre may be overcome by in-fibre derivitisation, or chemically fixing the absorbed analyte, but this work was beyond the scope of the present project.

At the end of the study, insufficient experimental results were obtained to provide estimates of the accuracy and sensitivity of the SPME-based TWA sampling method. However, the prototype field sampling system that was used is essentially simple. It consists of a custom-made fibre holder and a small sampling pump. Further research would be needed to overcome the problem of desorption of the analyte during the extended sampling period.

THE UTILITY OF ENZYME IMMUNOASSAY KITS (ELISA) FOR THE QUANTITATIVE AND SEMI-QUANTITATIVE IDENTIFICATION OF PESTICIDES IN WATER SOURCES TESTED FOR PESTICIDE POLLUTION

Enzyme-linked Immunoassays are tests based on the reaction of selective antibodies attached to solid supports with sensitive enzymes and analytes. They are capable of detecting low levels of pollutants with high specificity. For example, detection limits for chlorpyrifos vary from 0.1µg/L to 3.0µg/L and for endosulfan (as mixed isomers) from 0.08µg/L to 1µg/L. Immunoassay methods vary depending on the compound or compounds they are capable of detecting. The assays can be class-specific (they are able to screen for a certain class of compound), or compound-specific (they have a high specificity for the compounds of interest).

Two variations are commonly in use, one using an immobilizing antibody and the other an immobilizing conjugate on a solid-phase support such as a 96-well microtiter plate. The coating conjugate on the microtiter plate must differ from the immunogen to prohibit binding from antibodies that recognize the carrier protein. ELISAs for endosulfan and chlorpyrifos are in the form of plate kits. ELISA methods hold the promise of decreased sample processing, high compound specificity and significant increase in the number of samples that can be analyzed compared to traditional analytical techniques.

To evaluate the ELISA methods for measurement of endosulfan and chlorpyrifos, samples were tested in the laboratory to estimate the calibration

curve, and on field samples to compare ELISA methods to SPME (1 set of tests on samples from 9 field sites taken on the same day). In addition, a set of two triplicate analyses for endosulfan at 1ppb and at 0.1ppb respectively, were conducted to assess replicability at different concentrations.

Consistent detections by ELISA were made in the field samples. Contamination was comparable to that found in the previous study (WRC Report No. 795/1/ 2000). When comparing the ELISA method to the Solid Phase Extraction (SPE) method, qualitative agreement (present/absent) was high (8/9) for chlorpyrifos, but much lower (5/9) for endosulfan. One third of endosulfan results were false positives. Quantitative agreement between the two methods was weak. Triplicate analyses suggested good reproducibility for endosulfan estimation at both concentrations tested (range of difference = 15% at 1.0 ppb, and 7% at 0.1ppb).

From the tests performed, it appeared that the ELISA method could be useful as a semi-quantitative tool. Experience in using the ELISA for analysis indicated that the reagents were safe to use but were not as long-lasting, effectively limiting the value of the method unless there are high volumes of tests required. The ELISA method is therefore likely to be a useful screening tool for multiple samples as the method is not as time consuming as traditional methods of analysis.

THE COSTS ASSOCIATED WITH THE SPME, TWA AND ELISA PROCEDURES FOR MONITORING PESTICIDE WATER POLLUTION, AND COMPARISON TO CONVENTIONAL METHODS FOR MONITORING

To establish the utility of different monitoring methods for pesticide pollution, comparisons were made of the costs of the three analytical methods, *viz*.1) ELISA, 2) SPE and 3) SPME; and of the costs of the two integrated methods of sampling 1) 24-hour TWA SPME and 2) 24-hour auto sampling SPME. Comparisons in all cases were made in terms of the average cost of analyses per sample.

Standard econometric methods were used for the costing, which included estimation of direct costs (including capital costs such as equipment and recurrent costs such as labour) and indirect costs.

Of the three analytical methods, ELISA, SPME and SPE, the cost appraisal found SPME to have the lowest cost per sample (R253.86), followed by SPE (R329.86), while ELISA had the highest cost per sample, appreciably higher than SPME. For all three methods, the annualised capital cost was low and formed a low percentage (5-9%) of the total annual cost. Recurrent costs therefore formed the bulk of the costs of all three methods, with transport costs high for all three methods, and personnel costs for SPE and SPME being substantially higher than ELISA. The cost of materials were high for ELISA (R 240 per sample).

Sensitivity analysis showed that if the full cost of capital items (as totally dedicated to the analyses of the 384 annual samples) were included then the cost per sample for all methods increases substantially. ELISA would then rise to R450.66, for SPE to R429.86 and SPME to R502.01. In order to obtain the low cost per sample quoted above, 786 samples per annum are required for ELISA and 3200 samples per annum for SPE and SPME.

Despite an *a priori* expectation that the ELISA would be a useful method if there was sufficient economy of scale, costing analysis has shown that there is little to save with high volumes of tests since the highest costs for ELISA are not personnel but reagents. Moreover, given that SPME and SPE produce quantitative data with good detection limits (SPME 0.01-0.02 µg/L), and that the ELISA measurements are semi-quantitative, there appears to be little advantage to promoting the wider use of ELISA's at this stage.

The costs associated with using the autosampler compared to TWA for obtaining an integrated estimate for 24 hours were almost twice (R1707) that of the TWA derived from the SPME fibre (R865), largely because of the capital costs of the autosampler and time required to analyse each sample. The use of the autosampler in the field combined with laboratory SPME and GC analysis yields a quantitatively superior result compared to the use of the SPME fibre for in-field TWA sampling. However, it is evident that neither of these two methods are presently feasible for routine use in monitoring due to the logistics and costs associated with the collection and analyses of samples. Further investigation would be needed to establish their utility in improving the characterisation of rural water contamination before such high costs could be considered, or further refinements need to be developed to reduce costs.

SURVEY ON THE CAPACITY OF RURAL COMMUNITIES AND LOCAL AUTHORITIES TO IMPLEMENT MONITORING OF WATER SOURCES FOR PESTICIDES

In order to implement monitoring of pesticides in water, it is important to evaluate the capacity of rural local authorities in the Western Cape and other public sector responsible for water management.

The survey was intended to characterise the knowledge, perceptions and practices of rural target groups in relation to pesticide pollution of water, and to identify needs for training, technical and logistic support. A cross-sectional survey of farm workers, environmental health officers (EHOs), local authority management, rural NGOs and Government department staff was planned to meet these objectives. Questionnaire development, and field interviews of local authority officials and farm workers was undertaken by environmental health students from PENTECH supervised by 2 senior researchers from PENTECH and one from UCT. The survey was conducted during September and October 2001. For logistic reasons, local authorities and farms were surveyed separately, and different questionnaires were applied.

Final participants in the study included 8 EHOs. These participants were drawn from 7 rural farming districts in the Western Cape, which cover 21 towns with a total population of 503 000 From the management of water quality perspective, it was reported that in all 7 districts surveyed, water sources on farms are tested by EHOs. While the number of EHO's per district varies according to the population in the area, it is clear that in some areas there is a shortage of personnel. Only 3 (37%) of respondents felt that there were enough persons conducting water monitoring in their area. All respondents indicated that the costs of monitoring are borne by the local municipality, and only one respondent indicated that private farm owners may cover the cost if they request testing. Feedback of results of pesticide analysis was not common (only one respondent), while feedback to the farmer after request was relatively common (3/7 respondents).

The 63 farm personnel who participated in the study, included 6 sprayers (10%), 35 managers (57%) and 20 other workers (33%). They resided on 16 farms in 3 districts. Farm personnel reported their main water source for domestic use as being boreholes (25%), dams or rivers (44%), mountain springs (16%), rain water (19%) or reticulated water via municipal systems (19%). Unprotected sources for domestic water use was cited as canal water (13%), river or dam water (44%), and irrigation water (6%),). Farm personnel from 8 (50%) out of 16 farms indicated that water was tested on their farms. Four (50%) reported that water was tested for chemicals, 4 (50%) mentioned bacteria, 1 (13%) mentioned pH and 4 (50%) mentioned colour. However, the veracity of these reports could not be confirmed, and it is extremely unlikely that

pesticides are tested on 50% of farms in the region. This is borne out by responses from farm personnel when asked who carried out testing, they indicated mainly non-statutory bodies or individuals (6 of the 8 farms). Regularity of testing varied widely from once daily (n=1) to once yearly (n=2).

Although water monitoring was mentioned by respondents, a number of problems emerged. General monitoring appeared to take place relatively inconsistently in farming areas, and no monitoring for pesticides was reported. Although EHO's stated that they had a good knowledge of the health hazards of pesticides, there was a shortage of staff in many areas and there were not enough laboratories in the Western Cape to conduct pesticide water analysis. Lack of feedback to owners on results of analyses also appeared to be an area in need of intervention.

THE DEVELOPMENT OF GUIDELINES FOR PESTICIDE MONITORING OF RURAL WATER

A guideline document for use by rural communities and/or rural local authorities which sets out relevant aspects for conducting pesticide water monitoring is important in the context of this study which aims to develop screening methods for the pesticide monitoring of water. However, it was probably somewhat ambitious to try to produce both new technologies (methods for analysis, and instruments for integrating exposure in the field) as well as to recommend procedures based on these new technologies. For this reason, the project has been limited to recommendations on how a guideline document may be developed. It is based on the results of analyses of screening methods developed in Chapters 2-4, and the costs associated with these methods detailed in Chapter 5. The guidelines also reflect on the findings discussed in Chapter 6, in conjunction with the WRC project No. K5/795/00, of the capacity within rural communities to conduct water monitoring

The proposed guideline document would take into account factors which would optimise its utility in addition to content. Factors optimising the utility include physical structure and comprehensibility which should be undertaken with the assistance of language and media consultants. The content areas of the proposed guideline document should start by setting out a brief background on pesticide water pollution, with reference to WRC project No. 795/1/00 for additional information The guideline document will include sections covering the following:

1) purpose of the document
2) identification of target goups and potential applications
3) knowledge required before sampling for pesticides can take place
4) identification of areas and sites
5) identification of pesticides to be monitored.
6) procedures to follow and equipment required
7) methods available for analysis
8) selection of laboratories and analysis of samples
9) interpretation of results, and
10) management of water polluted by pesticides.

CONCLUSIONS

This project sought to establish new methods of pesticide analysis for monitoring of rural water sources, and to use these data to strengthen capacity for rural communities to monitor their own water sources. These objectives were only partly fulfilled in the course of the project. Clearly what has emerged is the utility of SPME fibres as a method of analysis for organic compounds, specifically two pesticides, chlorpyrifos and endosulfan, in water. The method produces cost-effective analyses of high sensitivity and repeatability.

Extending the use of the SPME fibres to produce a field device which would give an integrated estimate of water pollution has only been partly successful in this project.

A theoretical model of the rate of absorption of an analyte (pesticide) into the fibre coating, in a particular physical configuration, has been developed and tested using a prototype field device. Data produced in this project, though, has been too limited to draw any conclusions as to the validity or usefulness of the approach. Further research is recommended to overcome the problem of analyte retention during the extended sampling period, and to assess the importance of temperature control of sampling conditions in the field.

As for ELISA analysis, it is unlikely, based on the preliminary work conducted in this project, that ELISA analysis could become a useful adjunct in pesticide monitoring. Costs are high, much higher than anticipated in the literature, and accuracy weak. The use of ELISA even as a routine screening method could not be supported by the present associated costs.

Rural capacity to monitor pesticides remains limited, although awareness, as demonstrated in this and previous studies, is high. Costs are the chief barrier, and future work to reduce such costs will be the rate-limiting step for any widespread adoption of monitoring. However, the value of developing tools that give rural communities, local authorities, farmers and farm workers the capacity to initiate, coordinate, understand and interpret monitoring for pesticides in water, is highly desirable.

RECOMMENDATIONS

There is a need to develop pesticide monitoring methods with a view to the implementation of future full-scale monitoring. In particular, further development of the validity of the calculation adjusting for the loss factor, in-fibre derivation, and/or chemically fixing the absorbed analyte which was beyond the scope of the present project, should be explored in future research to establish integrated methods of monitoring pesticides.

Development of in-house plate kits which could substantially reduce costs associated with ELISA could be explored.

The preamble guidelines for monitoring pesticides in water developed in this study, should be further developed.

The capacity of State laboratories to measure pesticide water pollution at low levels (0.01 μg/L) using either SPE or SPME methods needs to be enhanced, especially if routine monitoring is implemented.

The problem of lack of feedback to farmers of the water results should be addressed.

It is important for farm residents to be informed about pesticide pollution of water and about the possible adverse health effects of pesticides. This could best be done by the farmer, farming co-op or supplier.

Chapter 1:

INTRODUCTION

CONTENTS

1.1 BACKGROUND

While the aim of ensuring a safe and reliable supply of water for all South Africans is the objective of the Department of Water Affairs and Forestry (DWAF), valid, reliable and cost-effective methods for monitoring water sources for pesticide pollution in South Africa remain elusive. The DWAF's current approach to water pollution is to combine uniform emission standards with pollution prevention by specifying the water quality requirements for receiving waters and specific water bodies. This approach takes into account the different assimilative capacities of individual receiving waters [1].

Although pesticide usage in South Africa is not as high as in developed countries or some developing countries, (South Africa uses less than Brazil, but more than other Latin American countries such as Chile, Peru and Ecuador[2]) it is the largest pesticide market in Sub-Saharan Africa, and the potential for environmental contamination is high[2-4]. For example, azinphosmethyl and endosulfan were recently detected in the Lourens River[5] and organochlorines have been identified in indigenous fish species in South African rivers[6]. While monitoring of water for pesticide pollution is a major enforcement activity in developed countries[7-9], relatively little takes place in South Africa at local government level, with most water quality monitoring focusing on microbiological contamination[10]. The majority of samples analysed at the State Laboratory in Cape Town are for pesticide residues in export fruit (K. Hershaw, Senior Laboratory Scientist, pers. comm.) However, there is emerging evidence that pesticide contamination of water even at relatively low concentrations is a health issue of concern[11,12] and is particularly related to the handling of pesticides on South African farms. Such exposures have increasingly been suspected of causing a range of adverse impacts on human health[12,13]. Despite this, methods for the detection of pesticides in water are currently constrained by a number of practical limitations:

a) Relatively few laboratories in South Africa undertake pesticide analysis and few provide details of rigorous quality assurance.

b) Those laboratories that do produce high quality analytical results are generally unable to do so on a large scale, or at a cost which would encourage widespread monitoring. It appears that testing for pesticide pollution is not presently cross-subsidised. Because of the high level of expertise and equipment required, the testing procedures involve reliance on expensive referral laboratories. Loss or degradation of samples en route could also be a problem. Such systems tend to force communities to be entirely reliant on outside expertise for solutions to local problems.

c) The capacity of local authorities and communities to manage pesticide pollution monitoring is presently unknown. However, given a culture in which little attention was paid to issues of water quality, as well as the prohibitive costs of analysis, it is unlikely that these authorities have the ability to carry out pesticide pollution monitoring, nor to interpret, or act upon, any results that might be obtained.

A previous study[14] undertaken by this research group investigated ground and surface water pesticide contamination in three intensive agricultural areas in the Western Cape; the Hex River Valley, Grabouw and Piketberg. Endosulfan and chlorpyrifos were extensively monitored and samples were also screened for other selected pesticides. At quantification limits of 0.1 μg/l endosulfan and 0.05 μg/l chlorpyrifos, the study found widespread contamination of both ground and surface water, as well as pesticide pollution of drinking water. The contamination was mostly at low levels, but regularly exceeded the stringent European (EU) Drinking Water Standard of 0.1 μg/l, and less frequently exceeded national

USA and California Environmental Protection Agency standards, which are 10 to 30 times higher[15-16] than EU standards. Some sampling sites were clearly "hot-spots" where significant levels were regularly detected. The two most contaminated sites were a sub-surface drain in the Hex River Valley and a dam in Grabouw, with 0.83 ± 1.0 μg/l (n=21) and 3.16 ± 3.5 μg/l (n=13) average endosulphan levels respectively. Other pesticides detected included chlorpyrifos, azinphos-methyl, fenarimol, iprodione, deltamethrin, penconazole and prothiofos. Endosulfan was detected more frequently in surface water (47%) than in groundwater (32%), while the detection of chlorpyrifos in ground and surface water was similar (55%). Contamination coincided mostly with irrigation, as opposed to spraying and trigger rains. Total dietary endosufan intake extrapolated from levels found in drinking water in the study areas, did not exceed the criteria of the Joint Food and Agricultural Organisation/World Health Organisation Meeting on Pesticide Residues (JMPR). The study showed that there is a need for monitoring pesticide contamination of surface and groundwater (and drinking water), and for the development of water quality standards in South Africa.

This project therefore builds on previous research[14], and attempts to address the questions listed above in 3 ways:

a. By evaluating two technological possibilities that could reduce costs and alleviate the practical obstacles to the analysis of pesticides in water.

b. Applying previous results, as well as findings from WRC Project No.795/1/00 to develop guidelines for stakeholder groups.

c. Identifying the human resource and technical support needed to enable stakeholder groups to implement pesticide pollution monitoring.

The development contribution of this project was intended to improve quality of rural life while at the same time to promote environmental justice and empowerment of rural communities. It is these vulnerable groups, rural communities and farm workers, who are most at risk from undetected and uncontrolled pesticide exposures in water.

1.2 AIMS AND OBJECTIVES

The aim of the project was to evaluate cost-effective methods for monitoring pesticide pollution in rural water.

The objectives were:

1) To evaluate the utility of solid-phase micro-extraction (SPME) fibres and programmable sampler as tools to achieve integrated sampling of water sources being tested for pesticide pollution.

2) Evaluate the utility of enzyme immunoassay kits for the quantitative and semi-quantitative identification of pesticides in water sources tested for pesticide pollution.

3) Develop a set of procedures/guidelines for use by rural communities and/or rural local authorities for monitoring water sources for pesticide pollution (based on the results of objectives I and 2, in conjunction with findings of the WRC Project No. 795/1/00[14]).

4) Estimate costs associated with the procedures evaluated under objectives 1 and 2, and compare these costs to conventional monitoring methods.

5) Produce a manual from procedures evaluated under objectives 1 and 2.

6) Evaluate the capacity of rural communities and local authorities to implement monitoring of water sources for pesticides, and make recommendations for strengthening such capacity.

1.3 REPORT OUTLINE

This report has been structured so that each chapter records in detail different objectives of the study.

The first chapter sets out background information; outlines the aims and objectives; explains the format; gives an outline of the report; and describes the study area, sites and pesticides analysed. Sampling issues are consistent throughout all chapters.

Chapters 2, 3 and 4 of the report give a detailed account of the literature, methods, results, and discussion of the findings, in order to evaluate:

a) The utility of solid-phase micro-extraction (SPME) fibres fo measuring pesticide contamination in water (Chapter 2)

b) Time Weighted Average (TWA) using SPME and a programmable sampler to achieve integrated sampling of water sources being tested for pesticide pollution (Chapter 3).

c) The utility of enzyme immunoassay kits for the quantitative and semi-quantitative identification of pesticides in water sources tested for pesticide pollution (Chapter 4).

These chapters therefore describe how objectives 1 and 2 were met, with Chapters 2 and 3 addressing objective 1 and Chapter 4 addressing objective 3.

Chaper 5, in fulfillment of objective 4, estimates the costs associated with the SPME, TWA and ELIZA procedures for monitoring pesticide pollution, and compares these costs to conventional monitoring methods.

Chaper 6 fulfills objective 6 and reports on the survey performed to evaluate the capacity existing in rural communities and local authorities to implement monitoring of water sources for pesticides.

Chapter 7 reports on the preliminary work towards the creation of guidelines for use by rural communities and/or rural local authorities (for monitoring of water sources for pesticide pollution). The guidelines are intended to build on the results of objectives l, 2 and 6, in conjunction with findings of the WRC project No.795/1/00). However, the project was not able to finalize a set of guidelines (objective 3) and a manual (objective 5), due to the time required to develop analytical tools sufficiently robust for field application (objectives 1 and 2). Given that these sampling tools still require further development, the aim of producing a guideline and manual may have with hindsight, been too ambituous. However, a preamble to a guideline document was developed (Chapter 7 and Appendix 7.1) which may be useful for future guideline development and methodologies used in the study were summarised (Appendix 2.2, Chapters 3 and 4) for other researchers to make use of.

The final chapter (Chapter 8) describes the most important overall conclusions, and lists recommendations arising from these conclusions.

1.4 STUDY AREA SITES AND PESTICIDES SELECTED IN THIS STUDY

The study area, sites and pesticides have been extensively investigated by the research team in a previous study[14].

The Hex River district (Figure 1.1) is an intensive grape-farming area in the Western Cape with a Mediterranean to semi-arid climate and moderate winter rainfall. This area was chosen for investigation because of high pesticide use. Water drawn from a nearby mountain dam is the most important source for drinking as well as for irrigation. The Hex River, which flows through the farming area, is vulnerable to pollution. Soil conditions encourage pesticide contamination of groundwater (shallow water table, unconfined aquifer, coarse soils with low clay content) as well as promoting surface water pollution.

The selected sites are summarised in Table1.1 and Figure 1.2. These sampling points included 3 along the course of the Hex River (E, F and G), representing a sequential flow downstream. No samples could, however, be taken from point E, because of low river flow at the time of sampling. Point G was from river water after the confluence of the Hex River and a tributary draining pristine mountain water from a northerly direction (see Figure 1.2). Other points included two reservoirs situated alongside vineyards (Ar and Bdr). Both are open tanks receiving water from a protected mountain spring. One reservoir (Ar) is situated in close proximity to a labourer's house. Two drains in the vineyards (Bdr and Cdr) that collect water from soil surface run-off and seepage were also included. Cdr is a collection point for an open drain that discharges both direct surface run-off (during heavy rain and irrigation, similar to a stormwater drain), as well as water moving through soil under the surface. Bdr is a collection point for a set of underground pipes that act as a sub-surface drain, collecting seepage through the soil in the vineyards at a depth of approximately 1 m. Both drains eventually feed into the Hex River. The drain Bdr is enclosed and flow was maintained throughout the year. Drain Cdr is open, and flow ceased during the dry summer months.

Based on previous experience, the pesticides endosulfan and chlorpirifos were chosen for analysis in the study. A more detailed description of the selection of study areas, sites and pesticides is given in the previous report[14].

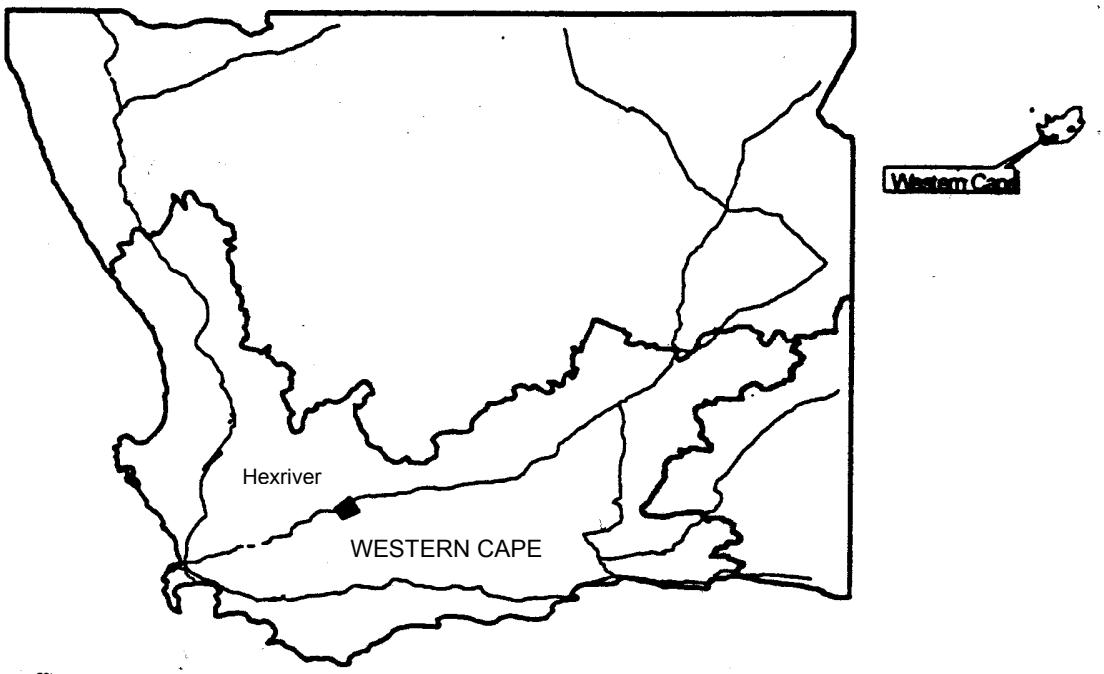

Figure 1.1 Location of area for pesticide sampling in the Western Cape, South Africa

Table 1.1: Sampling points in the Hex River Valley

Sampling point	Description
E	River point – high up the valley, towards the top of the production area
F	River point - in the middle of the river's course through the valley, in intensive agricultural area
G	River point - at lowest end of the valley, after confluence with a river from pristine area
Ar	Reservoir on farm: Spring water and mountain is source, but reservoir is open
Br	Reservoir on farm: Mountain is source, but reservoir is open
Cdr	Surface drain; Drains superficial vineyard run-off; Open drain
Bdr	Surface drain; Drains vineyard run-off and run-off from neighbouring farms; Closed drain.
Dd	Open farm dam

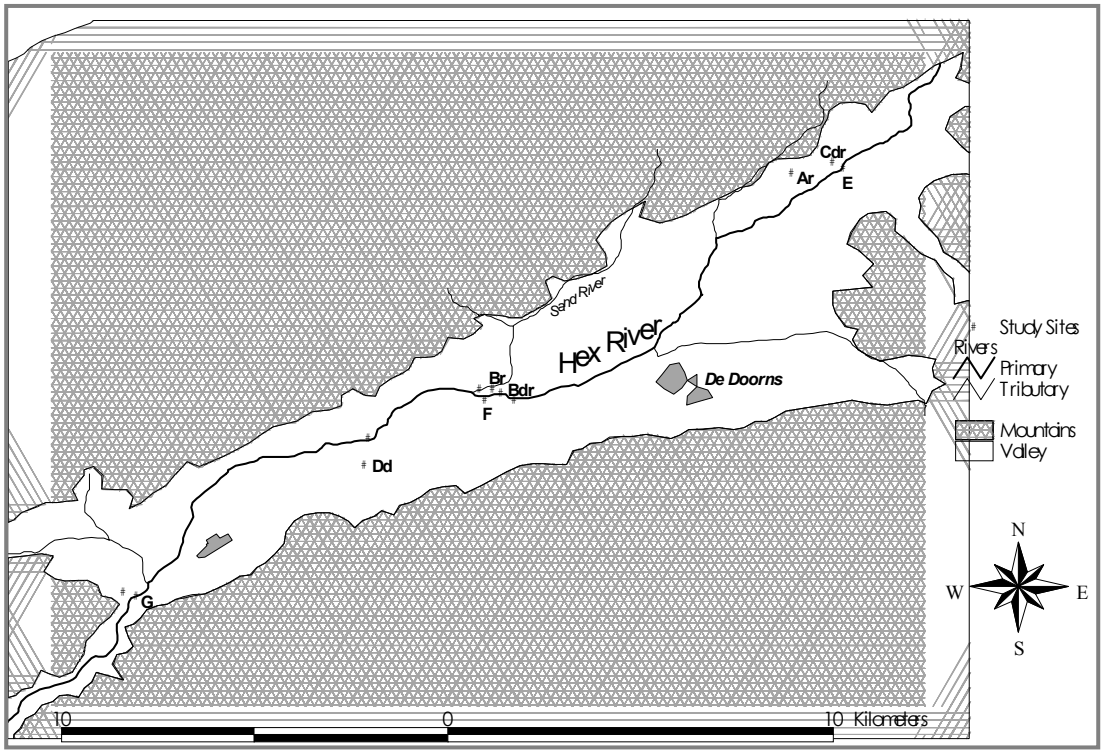

Figure 1.2: Location of sampling points for pesticides in the Hex River

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Chapter 2:

THE DEVELOPMENT AND ESTABLISHMENT OF THE SOLID PHASE MICROEXTRACTION (SPME)/GAS CHROMATOGRAPHY (GC) METHOD FOR THE ANALYSIS OF PESTICIDES IN CONTAMINATED STREAMS

CONTENTS

2.1 INTRODUCTION

In general, most organic pollutants of interest in aqueous environmental samples have to be extracted and enriched before instrumental determination of analyte concentrations. This isolation of the analyte from a sample is often achieved by sampling and extraction steps separate from the instrumental analysis (Eisert *et al*, 1996)[1]. Determination of pesticides by chromatographic techniques, such as Gas Chromatograph (GC) analysis, requires an extensive and time consuming step of sample preparation that usually includes the extraction step and a cleaning-up procedure in order to obtain a final extract fully compatible with the chromatographic determination (Beltran *et al*, 2000)[2].

To extract 1µg/l of an analyte from a 1 litre sample requires about 25ml of solvent, or the ratio of solvent to analyte of about $25X10^6$:1. The cost of the high purity solvents is significant. The discharge of these solvents into the environment is of concern as well (Cairns *et al*, 1992)[3]. In the last few years, several papers have outlined the need for a major simplification of sample preparation procedure. One approach was the miniaturization in scale of the sample extraction procedure (microextraction), resulting in a reduction of time and solvent consumption (Wan *et al*, 1996)[4]. The introduction of microextraction methods reduced the problem of chemical emissions into the environment and reduced costs as well. The microextraction method reduced the solvent and chemical usage for the extraction to 1/10 to 1/100 of the amounts used in macro methods (Cairns *et al*, 1992)[5].

The Solid Phase Extraction (SPE) method is known to be simpler and less time-consuming than classical extraction techniques, which it replaced, as many samples can be enriched in parallel. Later, it was found that an optimized selectivity could be achieved by using analyte-specific sorbents for different compounds. By coating the sorbent on a fine rod of fused silica (a fused-silica column, where the polymeric film is on the outside), the limitations of SPE were overcome. This extraction technique, termed Solid Phase Microextraction was introduced in the late 1980's (Pawliszyn *et al*, 1989)[6]. In 1990, Pawliszyn reported the first SPME device. The device was commercialized in 1993 by Supelco, together with the coated fibres used for the extraction (Beltran *et al*, 2000)[7].

Solid Phase Microextraction (SPME) was thus developed to address the need for rapid sample extraction and preparation in the laboratory. SPME is based on an immobilized liquid phase (for example, Polydimethylsiloxane - PDMS) as a stationary phase, and is used for the direct extraction of organic compounds from water by simply dipping the fibre into the aqueous sample (Eisert *et al*, 1996)[8]. The coated SPME fibre is attached to a metal rod, and a metal sheath that covers the fibre when not in use protects both.

Note that it is the polymeric coating that absorbs the analyte rather than the silica fibre, although the literature frequently refers to absorption (or adsorption) into the *fibre* rather than the *fibre coating*. This convention – use of 'fibre' rather than the more accurate phrase 'fibre coating' - will be followed in this report.

SPME has the advantage over SPE in that solvents are completely eliminated; blanks are greatly reduced, the procedure is simple and exhibits a degree of high linearity for many analytes (Abdel-Rehim *et al*, 2000)[9]. The extraction periods may also be greatly reduced (Eisert *et al*, 1996)[10]. SPME also has potentially better precision, lower detection limits and lower cost compared to SPE. The SPME method is relatively insensitive to matrix effects if standard parameters, such as pH, are controlled. Therefore, relatively high concentrations of other organics do not interfere with extraction. Another advantage of SPME is that the fibres can be used repeatedly, whereas with SPE the activated carbon cartridge is discarded after

use (Eisert *et al*, 1996)[10]. As mentioned before, it is a simple sample preparation method for gas and liquid chromatography (GC and LC) (Pawliszyn *et al*, 1992)[11].

Although the introduction of SPME was first referenced in 1989, the first applications on pesticide determination appeared in 1994 by Eisert *et al*[1]. Beltran *et al*[2] estimated that of about 400 references on SPME, approximately 60 dealt with pesticide residue analysis. Among the different chemical classes of pesticides, organochlorine and organophosphorous have received major attention over the past few years (Beltran *et al*, 2000)[12]. Most papers have dealt with the application of SPME to the determination of pesticide residue in water samples, not only because of the environmental relevance, but due to the fact that the technique fits perfectly for extraction of aqueous matrices (Beltran *et al*, 2000)[12]. The majority of applications for SPME are in aqueous medium and very few in solid medium.

In SPME, the fibre is inserted into the sample, usually contained in a capped glass vial, and the analyte absorbs into the fibre coating. At 'infinite time' the system reaches a dynamic equilibrium between the analyte in solution and that in the fibre. The distribution ratio between liquid (sample) and polymeric phases is a measure of the position of the equilibrium in the absorption process. The distribution constant may be a function of the concentration of the solute, chemical composition of the solute and the polymeric coating, pH, the temperature and the constituents of the sample (Zhang *et al*, 1994)[13]. The fibre is then removed from the sample and directly inserted into the sampling port of the Gas Chromatograph (GC), where it is thermally desorbed (i.e. a separation or extraction process that removes mixed organic contaminants using a thermal source) to determine the mass of the analyte.

Quantitation in water analysis by SPME is carried out by a calibration using external standards prepared with ultrapure water adding a minimum volume of pesticide standard solution and extracting them in the same way as a sample. There is a vast number of applications of SPME for the analysis of different pesticides types in water samples. Results from the first inter-laboratory study on pesticide analysis by SPME conducted in 1996, indicated that SPME is an accurate and fast method of sample preparation and analysis. A more recent inter-laboratory study confirmed these statements and added that the results proved that SPME is a reliable technique for quantitative analysis of the pesticide group studied in water (Beltran *et al*, 2000)[14].

Several papers deal with pesticide determination in more complex samples such as food samples (e.g. fruit and juices), soil samples and biological fluids (e.g. urine, serum and blood). When samples other than water are analysed, in most papers it is suggested that some sample pre-treatment be performed in order to simplify a sample matrix (Beltran *et al*, 2000)[15]. SPME has been used in many applications. It has been used in the extraction of polycyclic aromatic hydrocarbons (PAHs) in water (Lee *et al*, 1997)[16]; in the analysis of human breath (Pawliszyn *et al*, 1997)[17]; and to determine tetraethyl lead (TEL) and inorganic lead in water (Pawliszyn *et al*, 1996)[18].

Phenols have been monitored in industrial and municipal wastewaters and extracted from these waters using SPME. SPME has also been used for pharmaceutical applications (Dekker *et al*, 1999)[19]. The analysis of nonionic surfactants (soluble compounds that reduce the surface tension of liquids, or reduce interfacial tension between 2 liquids or a liquid and a solid), comprising commercial detergents, has also been done using SPME. The surfactants have been linked to effects on the human endocrine system and have been detected in sewage treatment plant effluents.

SPME has been used in the field of fuel related hydrocarbons, particularly total petroleum hydrocarbons (TPH) analysis. This is because the polydimethylsiloxane (PDMS) fibre is an excellent extraction medium for virtually all non-polar hydrocarbons in both water and air.

Researchers have also applied other analytical configurations to the determination of familiar compounds i.e. volatile organics have been detected using infrared spectroscopy (which is the use of the absorption, emission, or scattering of electromagnetic radiation by atoms or molecules to qualitatively or quantitatively study the atoms or molecules, or to study physical processes) (Dekker *et al*, 1999)[20].

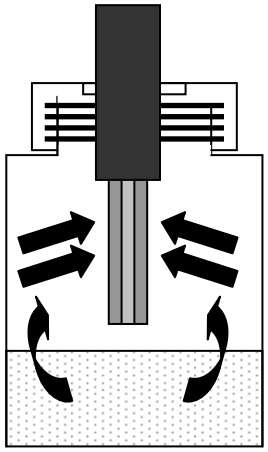
For this project, the utility of the SPME fibres was evaluated as an alternative, cost-effective method for monitoring of water systems for pollution by pesticides (London *et al*, 1999)[21]. The SPME method was evaluated by establishing the optimum conditions for the sample extraction, assessing the reproducibility and determining the Method Detection Limit (MDL). Extensive laboratory assessment of the SPME fibres was conducted for the recovery and analysis of chlorpyrifos and endosulfan, the main pesticides under investigation. Field samples were analysed to confirm that the method could be applied to environmental samples.

2.2 THEORY

SOLID PHASE MICROEXTRACTION THEORY

Transfer of the analyte from the sample to the SPME fibre is an equilibrium process, thus analytes are not completely extracted from the matrix. The liquid polymeric coatings provide a non-exhaustive liquid-liquid or gas-liquid extraction. When a sample is placed in a closed vial, equilibrium forms between 1.) fibre coating to aqueous phase (i.e. water sample), 2.) Headspace (the space between the surface of the liquid and the vial cap) to aqueous phase, and 3.) fibre coating to headspace (Figure 2.1). Analyte recovery is related to overall equilibrium of the 3 phases present in the sampling vial.

Figure 2.1: Diagram of an SPME fibre performing headspace sampling (Pawliszyn, 1997)[22]



The distribution of analyte among the 3 phases, after equilibrium, is given by Equation 1 (Dekker, 1999):

$$C_0V_S = C_H^iV_H + C_S^iV_S + C_f^iV_f \qquad \text{.....(1)}^{23}$$

C_0 is the initial concentration of the analytes in the aqueous solution; C_H^i , C_S^i and C_f^i are the equilibrium concentrations of the analytes in the headspace, aqueous solution and fibre coating, respectively. V_H, V_S and V_f are volumes of headspace, aqueous solution and fibre

coating, respectively. It should be noted that if no headspace exists in the closed vial, then the headspace term ($C_H^i V_H$) is eliminated and the equilibrium is solely formed between the fibre and aqueous solution.

Partitioning between the fibre coating and the aqueous phase is described by a distribution constant (K_{fs}) (Pawliszyn, 1997; Dekker, 1999):

$$K_{fs} = C_f / C_s \qquad \ldots\ldots\ldots(2)^{24}$$

Where, C_f is the concentration of analyte in the fibre coating and C_s is the concentration of the analyte in the aqueous phase. K_{fs} is the parameter that describes the properties of the fibre coating and its selectivity toward a specific analyte in comparison to other matrix components.

The partition ratio (k') is defined as follows (Dekker, 1999):

$$k' = C_f V_f / C_s V_s = m_f / m_s = K_{fs} V_f / V_s \qquad \ldots\ldots\ldots(3)^{24}$$

In equation 3, m_f and m_s are the number of moles in the fibre coating and aqueous phases, respectively. The V_f and V_s are volumes of the fibre coating and the sample respectively. The PDMS coatings used for SPME have strong affinities for organic compounds, therefore K_{fs} values for targeted analytes are usually large and the SPME has a very high concentrating affect and leads to good sensitivity. For example, for a PDMS coated fibre the distribution constants are as follows (Pawliszyn, 1997)[27]: endosulfan-alpha: K_{fs} = 25000, endosulfan-beta: K_{fs} = 10000, endosulfan sulfate: K_{fs} = 400

When the aqueous sample volume (V_s) is much larger than the stationary phase volume (V_f), the following equation is used (Dekker, 1999):

$$m_f = K_{fs} V_f C_s^0 \qquad \ldots\ldots\ldots(4)^{28}$$

Where, m_f is the amount extracted by the fibre coating. In general, for comparatively large sample volumes (usually > 5ml, but larger volumes may be necessary for high K_{fs} values), the amount of analyte absorbed by the fibre coating at equilibrium is directly proportional to the initial aqueous concentration, C_s^0 and equation (4) may be used. (The exact sample volume does not have to be known, ideal for field sampling and simplification of laboratory operations).

When sampling from a finite volume, the sample can significantly deplete – the fibre may extract sufficient analyte to reduce the concentration of the remaining sample. The equation to be used to estimate the mass extracted m_f is as follows (Dekker, 1999):

$$m_f = K_{fs} V_f V_s C_s^0 / [(K_{fs} V_f) + V_s] \qquad \ldots\ldots\ldots(5)^{29}$$

DETERMINATION OF THE DISTRIBUTION CONSTANT (K_{FS}) VALUES

The distribution constant (partition coefficient) is defined by the relationship $C_s = K_{fs}.C_f$, at equilibrium. The time required to approach equilibrium values depends on the Biot Number. The time required to achieve 95% of equilibrium may be 1 hour for sufficiently large Mass Transfer Biot numbers (refer to Chapter 3), or several days for sufficiently small Mass Transfer Biot Numbers (less than 0.3). For the sampling system consisting of the fibre of thickness 100um fully exposed in the sampling vial, under vigorous stirring, the theoretical

Mass Transfer Biot numbers > 20 are predicted for a range of K_{fs} values. However, the time required to approach equilibrium was determined empirically rather than theoretically, and was found to be about 2 hours for the analytes under consideration. Thus the K_{fs} values were estimated by exposing the fully extended fibre in a vial containing 1 µg/L mixed standard solution, under vigorous stirring, for a period of 3 hours.

Previous experimental work demonstrated that the arbitrarily chosen standard concentration of 1µg/L used in these experiments was well above the Method Detection Limit (MDL), and that good reproducibility of analytical results – the measurement of analyte mass (or concentration) on the fibre - could be achieved. The mass of analyte absorbed (the absolute extracted amount) was estimated by comparing the GC response to a calibration curve based on direct injection of a series of standards, that is, by external calibration. Although this is the generally accepted method for measuring the absolute extracted amount, the method has been criticized due to the differences between thermal absorption and liquid injection/vaporisation (Tuduri *et al*, 2000)[30]. The known fibre volume is then used to calculate the concentration in the fibre, and hence the K_{fs} values.

The variation of K_{fs} values with temperature is given by (Pawliszyn, 1997)[31]:

$$\frac{K_{fs1}}{K_{fs2}} = \exp\left[\frac{-\Delta H}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right] \qquad \text{.....} \qquad \textbf{(6)}$$

In this equation, K_{fs1} and K_{fs2} refer to the values at absolute temperatures T_1 and T_2; ΔH is the molar enthalpy change on analyte absorption into the fibre and R is the gas constant. As ΔH is essentially constant over the temperature ranges applicable to SPME work, the percentage change in K_{fs} values with temperature may be estimated using this equation.

K_{fs} is affected by extraction conditions such as salting, pH and temperature. Therefore, when sampling outdoors, the temperature effect must be considered when temperature variations occur. The effect of temperature must be considered when heating is used to increase sample extraction rate as well as to enhance the release of analytes. By using equation 6, a scenario demonstrating the effect of temperature may be illustrated. When a K_{fs} value for an analyte is greater than 1, the ΔH is greater than zero. Equation 6 shows that an increase in temperature should decrease the K_{fs} value (Pawliszyn, 1997)[32].

AGITATION OF SAMPLE

Agitation (mixing) of the sample accelerates the rate of mass transfer from the sample to the fibre - effectively reducing extraction times by decreasing the time required to approach equilibrium. No mixing occurs in the stagnant boundary layer adjacent to the fibre, but mixing occurs in the rest of the sample. Mass transfer occurs slowly through the boundary layer. The reproducibility of results is improved if the rate of mixing (usually controlled by the rotational speed of the magnetic stirrer) and the position of the fibre in the vial are the same from sample to sample. After a specified elapsed absorption time, the fibre is retracted back into the sheath and removed from the sampling vial. The sheath is inserted into the injection port of the GC and the plunger is lowered to expose the fibre to a high temperature, where the analytes are thermally desorbed.

KINETICS OF THE EXTRACTION PROCESS

The (analyte) mass transfer process involves the following: 1.) Diffusion (in general, by convective and/or molecular processes) through the liquid sample, and 2.) molecular diffusion into the fibre coating. One or both processes may be rate-limiting, depending on the

sampling conditions, the physical properties of the materials in the system and the dimensions of the system.

The time required for the concentration of the analyte in the fibre coating to approach equilibrium is determined by the rate of mass transfer from the sample to the fibre, and diffusion within the fibre coating. In an agitated system, mixing in the bulk of the liquid is rapid. Mass transfer by molecular diffusion through the boundary layer adjacent to the fibre is a function of the boundary layer thickness. Rapid stirring reduces the boundary layer thickness and hence reduces the absorption time. Mass transfer from the surface of the coating to the interior is rapid due to the short transfer path (100 μm) and the (usually) high K_{fs} values.

In liquids, diffusion coefficients (diffusivities) are several orders of magnitude lower than in gases. Thus rapid extraction of the analytes in aqueous media requires some form of agitation in order to reduce the boundary layer thickness. Without agitation, extraction time may take hours, and the mass extracted may vary considerably from one analysis to the next (for the same analyte concentration) if there is a lack of control over the flow conditions in the vicinity of the fibre.

The system being used for the experimental laboratory testing may be modelled as an unsteady state problem.

By optimizing the position of the fibre in a stirred sample, the extraction time may be shortened. The thickness of the boundary layer can be estimated from the empirical formulae of fluid mechanics. If the fibre is placed off-centre in the vial so that the fluid flows past the fibre at an angle to the fibre axis, the boundary layer thickness can be estimated using Equation 7 (Pawliszyn, 1997):

$$\delta = 9.52\, b/(R_d^{0.62} Sc^{0.38}) \qquad \text{…….(7)}^{33}$$

Where, R_d is the Reynolds number ($R_d = 2ub/v$, where u and v are the linear speed of the fluid and kinematic viscosity), b is the coated fibre radius and Sc is the Scmidt number of the liquid (equaling v/D, where D is the diffusion coefficient of the analyte in the liquid.

The tangential velocity in water agitated by stir bar in a cylindrical container is predicted by using Equation 8 or Equation 9 (Pawliszyn, 1997):

$$u(r) = 1.05\pi Nr[2 - (r/0.74R)^2], \text{ for } 0 < r \leq 0.74R \qquad \text{…….(8)}^{32}$$

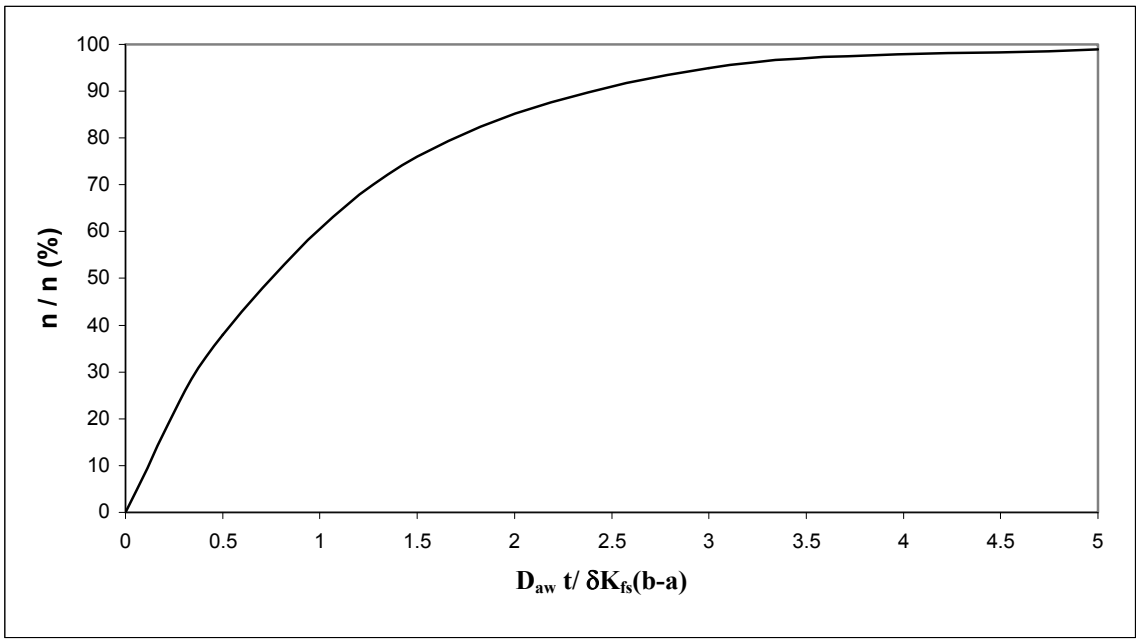
$$u(r) = 0.575\pi NR^2 1/r \qquad \text{…….(9)}^{32}$$

Where, R is the radius (half-length) of the stir bar and N the revolutions per second.

Equations 8 and 9 may be used to estimate the tangential velocity at a given radial position r, and the radial position at which the maximum tangential velocity occurs.

Figure 2.2 is a schematic diagram showing the approach to equilibrium.

Figure 2.2: Rate of approach to equilibrium vs boundary layer thickness (Dekker, 1999)[34]



In Figure 2.2, the y-axis is the percentage of total analyte mass absorbed by the fibre at equilibrium. As the time increases, the mass absorbed increases. Time is represented as a dimensionless unit, on the x-axis, of the analyte diffusion co-efficient in the sample fluid (D_{aw}), divided by the boundary layer thickness (δ), the distribution constant between the fibre and sample (K_{fs}), the fibre coating (b-a) where 'a' is the fibre coating inner radius and 'b' is the fibre coating outer radius, and the time to reach equilibrium.

Figure 2.2 indicates that equilibrium is approached asymptotically, implying that the system requires 'infinite' time to reach equilibrium. A change in mass extracted cannot be determined if it is smaller than the experimental error (usually about 5 %). The equilibration time is dependent on the K_{fs}, the analyte diffusion co-efficient in the coating. Also, the extraction time will be longer when using thicker fibre coatings because analytes must travel longer and further to completely penetrate the coating. A thicker coating may be chosen to improve sensitivity, but an increase in extraction time may occur. Lastly, a thicker boundary layer will increase extraction time.

An approximation to the equilibration time, the time required to reach 95% of the equilibrium concentration, for an agitated solution with a boundary layer can be determined using the following equation (Dekker, 1999):

$$t_e = t_{95\%} = 3\delta K_{fs}(b\text{-}a) / D_{aw} \qquad \text{.....(10)}^{33}$$

Where, δ is the boundary layer thickness, K_{fs} is the analyte's distribution constant between the fibre and sample, *(b-a)* is the fibre coating thickness and D_s is the analyte diffusion co-efficient in the sample fluid. This equation may be used to estimate the equilibration time when the extraction time is controlled by the diffusion of the analyte through the boundary layer.

GAS CHROMATOGRAPHY BASIC THEORY
GAS CHROMATOGRAPHY (GC)

Gas chromatography is a sensitive and selective method for the qualitative and quantitative determination of substances that are stable in the vapour phase. Gas chromatography is based on the phenomenon that occurs when a mixture of volatile materials is transported by a carrier gas eluent through a column containing an absorbing material coated on a solid material. Each volatile component is partitioned between the stationary phase and carrier gas. The length of time required for a volatile analyte to pass through the column depends upon the degree to which it is retained by the stationary phase. The time at which an analyte emerges, and its quantity may be measured via suitable detectors.

The gas chromatograph consists of different components. The carrier gas is a gas constituting the mobile phase. It must be very pure and is generally argon, helium, nitrogen or hydrogen. This gas must be chemically unreactive toward the sample and chromatograph components. A sample is injected as a single compact plug into the carrier gas stream immediately ahead of the column entrance. If the sample is liquid, it is important to heat the injection chamber to vapourize the liquid quickly, otherwise tailing occurs, which in turn cause spreading of the peak. If the sample is heated too much, thermal decomposition may occur and produce inaccurate results.

The column, through which the carrier gas and analyte species flow and in which separations occur, is the key component of the gas chromatograph. Superior results are usually obtained with a capillary column consisting of a very long, small-diameter quartz tube with the stationary liquid phase coated on the inside of the column. A column is usually chosen for its ability to separate compounds in a group of compounds from each other.

A component that primarily determines the sensitivity of gas chromatographic analysis, as well as the selectivity, is the detector. The detector shows when an analyte peak is emerging from a column and responds to the quantity of the analyte in the peak.

ELECTRON CAPTURE DETECTOR (ECD)

The principle of the ECD is that some organic compounds take up electrons in an ionized gas (particularly those compounds containing chlorine, and fluorine), changing a current flowing through the gas. With the ECD, the carrier gas is passed over material containing a radioactive element that emits electrons (β-emitter). β-Emitters used for this purpose may be nickel-63 or tritium adsorbed on platinum or titanium foil. Electrons from the β-emitter ionize the carrier gas causing more electrons to be released. The carrier gas is then electrically conductive. When a solute that is capable of picking up electrons, is present in the carrier gas, the number of free electrons is decreased and the current is therefore decreased.

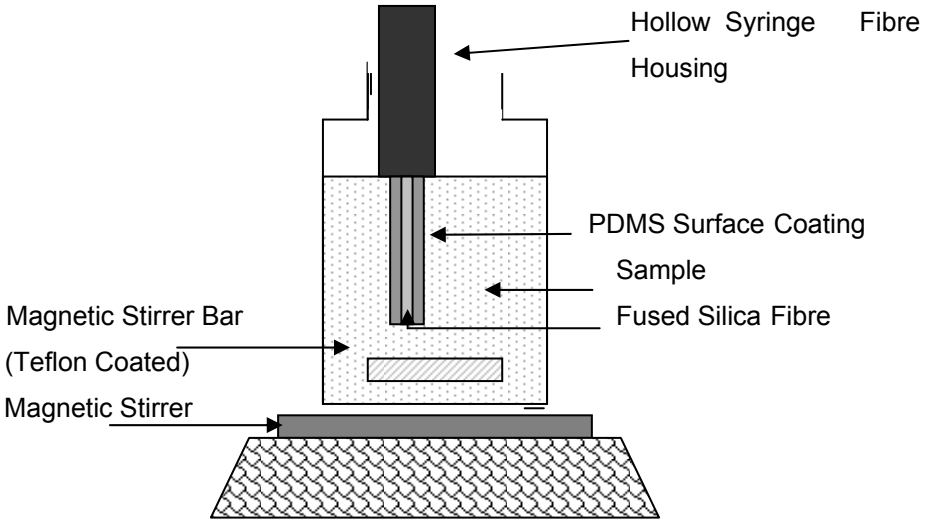
ECD has an insensitivity toward hydrocarbons, which means that ECD-detectable compounds may be measured in high-hydrocarbon backgrounds. ECD does not respond to either alcohols or amines. The detector is non-destructive and sample peaks may therefore be collected after detection (Manahan, 1986)[35].

2.3 MATERIALS AND METHODS

2.3.1 REAGENTS AND EQUIPMENT

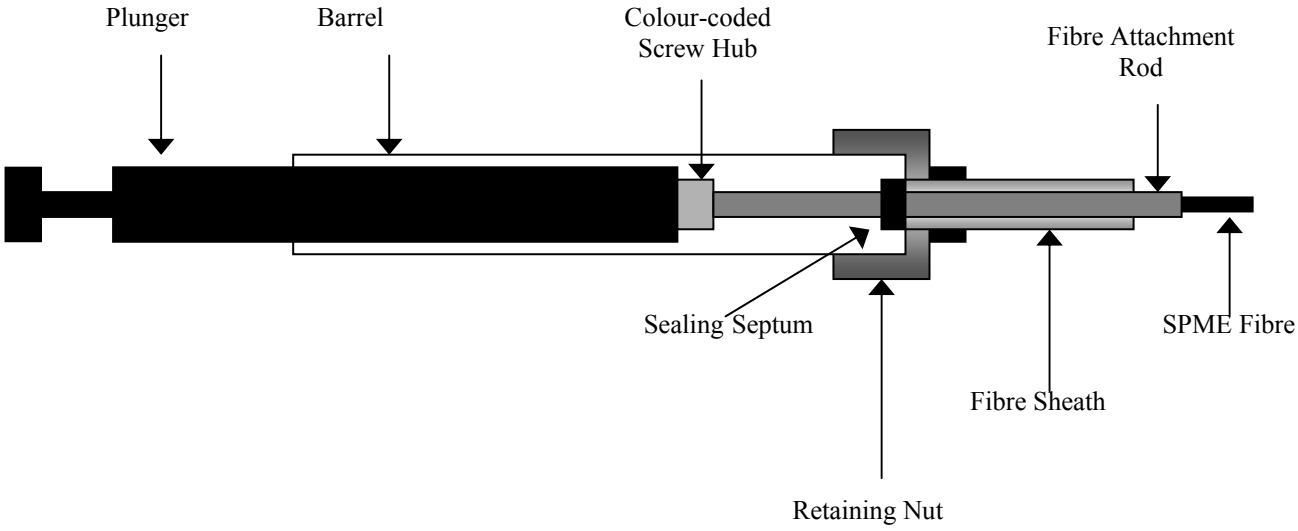
SPME utilizes a short, thin, solid rod of fused silica that is coated with an absorbent polymer (Polydimethylsiloxane - PDMS). The length of the fused silica fibre is usually 10mm long, with coating thickness of 7, 30, 65, 75, 85, or 100μm. The coated SPME fibre is attached to a metal rod. A protective metal sheath covers the rod and fibre when retracted. The 100μm fibre was the coating size specification that was chosen for the experimental work. The SPME fibres were supplied by Supelco and distributed by Anatech. Chlorpyrifos and endosulfan (alpha, beta, sulfate) standards, in crystal form, were supplied by Chem Service Inc. Analytical Grade Methanol was used as the solvent to prepare the standards and was obtained from PENTECH Analytical Laboratories. Glassware supplied by PENTECH Analytical Laboratories. A Heidolph MR3001K Magnetic Stirrer (Supplied by Labotec) was used, together with Teflon coated stirrer bar (Dimension: Length = 20mm; Diameter = 5mm), to stir the aqueous sample. The gas chromatographic analysis was performed using a Varian model 3300 Gas Chromatograph (GC), equipped with an electron capture detector (ECD). The Packed Column detector has a temperature range of 120°C to 420°C. The GC has an injector temperature of 170°C, Column temperature of 250°C and a Detector temperature of 300°C. The Delta chromatography data system (Version 5.0) was used for data processing (Supplied by Digital Solutions (Pty)(Ltd)). Nitrogen was used as a carrier gas and was supplied by Fedgas. The GC cycle time was set at 25 minutes; 22 minutes for the elution of the analytes and 3 minutes for the GC to stabilize and return to the original temperature settings after the temperature program had elapsed. The sample volume selected was 10.0ml. The type of sampling was aqueous not headspace. Prior to the first extraction, the fibres were conditioned at 290°C for 1 hour as was recommended by the manufacturer.

Figure 2.3: Diagram of an SPME fibre exposed to an aqueous contaminant sample



The fibre is placed off-centre, as illustrated in the diagram, at the position of maximum tangential velocity, and requires minimum extraction time i.e. extraction of the analyte from the sample (Pawliszyn *et al*, 1997)[32].

Figure 2.4: Schematic diagram of an SPME fibre with holder (Supelco supplied)



The SPME procedure involves the partitioning of analytes between the sample and the fibre coating and thermal desorption of the concentrated analytes from the fibre coating in the GC port. For the extraction procedure, an aqueous sample containing organic analytes is placed in a vial and sealed with a cap and septum. The SPME protective sheath pierces the septum and the plunger is lowered to expose the fibre directly to the aqueous sample. Analytes are then extracted from the sample matrix into the fibre coating. The time for the analyte

concentration in the fibre to approach equilibrium is determined by the mass transfer rate from sample to fibre.

The thickest available Polydimethylsiloxane (PDMS) SPME fibre was used for the majority of the experimental work reported. One reason for the choice of the thickest fibre coating is because the thicker the coating, the more analyte will be extracted and the lower the detection limit. The 100μm fibre retains a greater mass of the volatile analytes, therefore, it was selected as the fibre of choice to be used for the analysis. The PDMS coating was chosen because it has a high affinity for the pesticides of interest in this study, and was recommended in the commercial and scientific literature.

2.3.2 METHODS FOR SPME/GC METHOD

2.3.2.1 Determination of optimum absorption time

A series of experimental runs were performed at different absorption times in order to establish the optimum absorption time to be used. The runs were conducted using a magnetic stirrer (operating at 625 RPM) and in a temperature-controlled environment. The temperature of the environment was maintained at 20^{o}C. Each borosilicate glass vial contained a sample with a volume of 10ml. A spiked analyte solution (mixed standard) of known concentration 5 µg/L (parts per billion - ppb) containing all 4 analytes, namely chlopyrifos, endosulfan-alpha, endosulfan-beta and endosulfan sulfate, was used for the runs. The SPME fibre was fully exposed (10mm) to the spiked analyte solution (5µg/L concentration), under vigorous stirring for a range of absorption times each. These times were as follows (minutes): 5, 10, 15, 20, 25. These runs were replicated to provide an indication of the effect of absorption time on reproducibility.

2.3.2.2 Evaluating the effect of agitation

A series of laboratory runs were conducted to test the effect of agitation of the sample. The effect of agitation was tested on all 4 analytes, individually, as well as the effect on the reproducibility of results without stirring (agitation). There were 2 parts to the experiment. The effect of a complete absence of agitation on reproducibility was assessed by exposing the SPME fibre to the spiked analyte solution (5µg/L) in the borosilicate glass vial, for absorption times ranging from 5 minutes to 25 minutes. A set of experimental runs was then conducted with the SPME fibre exposed to a newly prepared spiked analyte solution (5µg/L), stirred at 625RPM, for absorption times of 5 to 25 minutes. In all cases, the fibre was exposed to its full length of 10mm; stirring (for the second set of runs) was achieved using a magnetic stirrer and a Teflon-coated magnetic stirrer bar located inside the glass vial containing the spiked analyte solution.

2.3.2.3 Reproducibility of the SPME method

The initial experiments (described in 2.3.2.1 and 2.3.2.2) were used to decide on standard experimental parameters for agitation rate and absorption time. In order to assess the overall reproducibility of the method, data for replicate runs done under standard conditions, including laboratory spiked standards and field samples, were collated and statistically analysed. The differences between each replicate set, for each analyte, were calculated. Thereafter, the mean of the differences for each analyte and the standard deviations of these differences was determined.

2.3.3 EQUILIBRIUM RUNS

The reproducibility of analyses depends primarily on reproducing extraction conditions - stirrer speed, temperature of the sample during extraction, position of the fibre and absorption time, rather than extracting for long enough to approach the equilibrium partitioning of the analyte between the fibre and the sample. Nonetheless, experimental runs were conducted to estimate the time required to approach equilibrium. The tests were done at a controlled temperature of 20^{o}C. The SPME fibre was fully exposed (10mm) to a spiked analyte solution, containing all 4 analytes, of concentration 1µg/L (ppb) for a range of absorption times ranging from 20 minutes to 140 minutes. The spiked analyte solution in the borosilicate glass vial was vigorously stirred at maximum, using the magnetic stirrer and Teflon-coated stirrer bar laboratory set-up.

2.3.4 DETERMINATION OF THE METHOD DETECTION LIMIT (MDL)

There are several approaches to defining and determining the Method Detection Limit. A widely accepted definition is based on using low concentration spikes into a blank matrix and calculating the standard deviation of 7 to 10 determinations. The Method Detection Limit (MDL) is then defined as 3 times the standard deviation obtained for the analyte concentration, for a concentration not higher than 10 times the MDL.[36] This definition and approach is similar to that used by the US EPA.

Thus: $$\mathrm{MDL} = \frac{3 * (\text{Standard deviation}) * (\text{Concentration in Solution [Cs]})}{\text{Mean Peak Area}}$$

A series of experimental runs were conducted in order to determine the Method Detection Limit of the SPME fibres. Initially, the MDL was unknown hence the value of 10 times the MDL was unknown. Three different (successively lower) concentration levels were therefore selected (i.e. 1.00µg/L, 0.04µg/L and 0.02µg/L) to determine the MDL, and seven repeat runs were performed at each concentration. The standard deviation was then determined for the seven runs at each concentration level.

2.4 RESULTS

SOLID PHASE MICROEXTRACTION (SPME) RESULTS

2.4.1 DETERMINATION OF 0PTIMUM ABSORPTION TIME

A series of experimental runs was performed at different absorption times in order to establish the optimum absorption time to be used. The runs were conducted with a magnetic stirrer, operating at 625 RPM, and in a temperature-controlled environment. The temperature of the environment was maintained at 20^{o}C. Each vial contained a sample with a volume of 10ml. A mixed standard of known concentration 5 µg/L (parts per billion - ppb) containing all 4 analytes, namely chlopyrifos, endosulfan-alpha, endosulfan-beta and endosulfan sulfate, was used for these runs. The SPME fibre was exposed to the mixed for a range of absorption times: 5, 10, 15, 20, 25 (minutes). Each run was replicated in order to simultaneously provide an initial assessment the reproducibility of the method. The results are given in Table 2.1.

Table 2.1: Range of absorption tmes for 4 analytes in a mixed standard –5µg/L (ppb)

	Chlorpyrifos		Endosulfan-alpha		Endosulfan-beta		Endosulfan Sulfate	
Absorption Time	Detector Counts	Differences	Detector Counts	Differences	Detector Counts	Differences	Detector Counts	Differences
(Minutes)	(uVSec)	(%)	(uVSec)	(%)	(uVSec)	(%)	(uVSec)	(%)
5	52871	0.7	93417	1.2	81295	9.4	53573	23.3
5	53250		92258		74017		67693	
10	106597	4.6	185283	7.3	136663	7.9	136954	3.3
10	111632		199417		147887		141519	
15	160836	18.9	298104	16.6	208106	13.9	190070	9.3
15	133091		252293		181132		173144	
20	161511	3.0	385601	0.7	230428	11.3	201886	3.5
20	166415		388437		205737		209095	
25	177905	3.9	420641	3.8	188768	19.3	211686	1.3
25	185009		436853		229008		208903	

The GC detector response (Detector Counts columns in Table 2.1) is proportional to the mass absorbed into the fibre, then thermally desorbed into the GC port. The percentage difference between replicate columns provides an indication of reproducibility at different absorption times.

Figure 2.5: Graph of detector counts versus absorption time for a stirred chlorpyrifos 5µg/L (ppb) standard

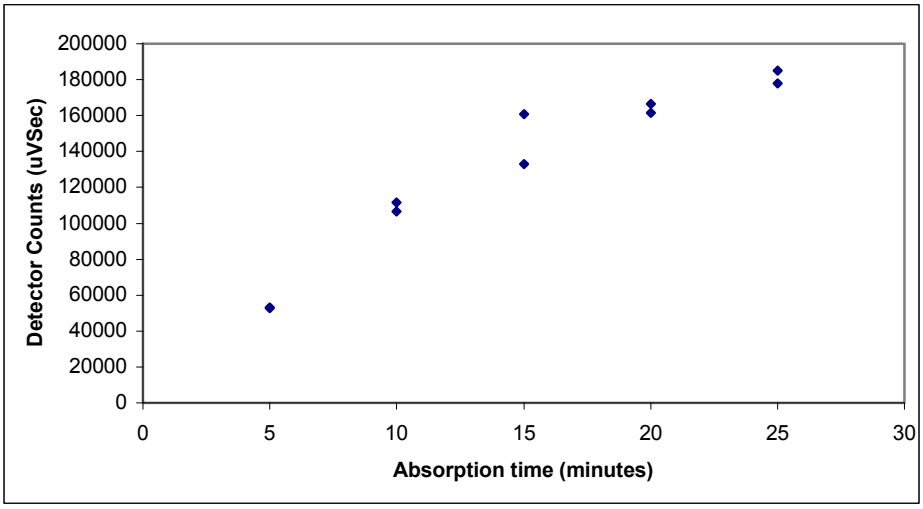
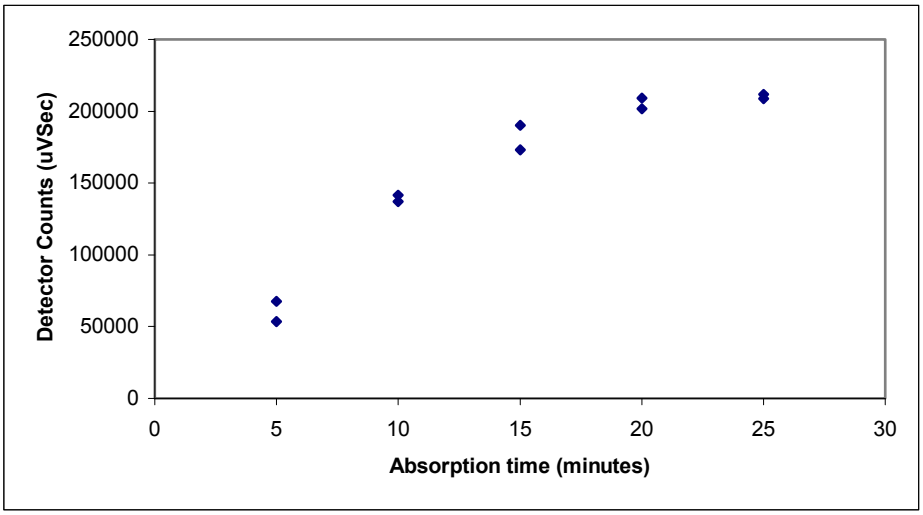


Figure 2.6: Graph of detector counts versus absorption time for a stirred endosulfan sulfate 5µg/L (ppb) standard



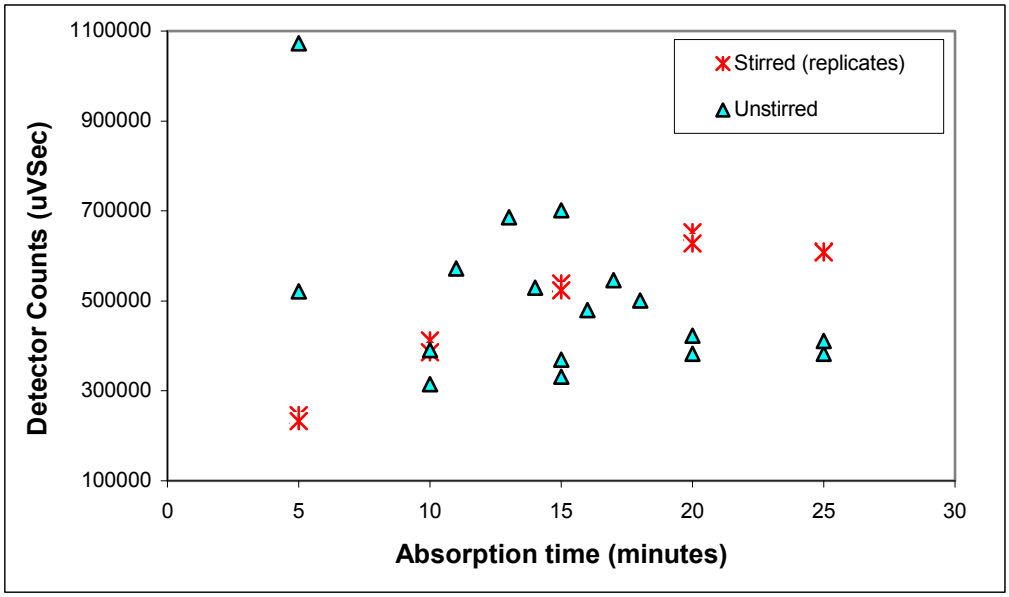
The graphs for endosulfan-alpha and endosulfan-beta are similar.

2.4.2 EVALUATING THE EFFECT OF AGITATION

A series of runs was conducted to test the effect of agitation of the sample on the reproducibility of analyses, over a range of absorption times of 5 to 25 minutes. The effect of agitation was tested on all 4 analytes, individually, as well as the effect on the reproducibility of results without stirring (agitation). The Figure 2.7 illustrates the behaviour of the chlorpyrifos standard at a concentration of 10µg/L (ppb). There were 2 parts to the experiment. The first part was exposing the SPME fibre to the chlorpyrifos solution in the glass vial, unstirred, at different absorption times (ranging from 5 minutes to 25 minutes). The second part was exposing the SPME fibre to a newly prepared chlorpyrifos solution, stirred at 625 RPM, over the same range of absorption times. A Magnetic stirrer and a Teflon-coated magnetic stirrer bar located inside the glass vial containing the analyte solution was used for stirring.

The results are shown in Figure 2.7:

Figure 2.7: Detector counts (proportional to mass absorbed) for a stirred and unstirred chlorpyrifos sample vs absorption time



Without agitation of the sample, concentration gradients may be expected within the sample, and the rate of mass transfer to the fibre may be expected to be erratic due to concentration differences within the sample and random eddies within the sample due to thermal and concentration gradients. Vigorous stirring of the sample results in good bulk mixing. The rate of mass transfer of the analyte from the sample to the fibre becomes a reproducible function of time. Figures 2.5 and 2.6 show that the Detector Counts (proportional to the mass absorbed) increases with time, towards an equilibrium value, as expected. For the unstirred samples, the rate of mass absorbed was erratic (large scatter in the data), particularly for absorption times less than 20 minutes. For longer absorption times, mixing due to thermal and concentration gradients tends to reduce the scatter. By stirring the sample, bulk mixing (uniform sample concentration) is rapidly achieved and the reproducibility between stirred sample replicates was very good.

2.4.3 REPRODUCIBILITY OF THE SPME METHOD

Samples from 3 Hex River Valley sites (sampled during the previous WRC Project 795/1/00) were obtained and analysed in replicate. The differences between these replicates, and the replicates of 17 laboratory mixed standards, were used to assess the overall reproducibility of the SPME method with respect to each of the analytes. The results are presented in Table 2.2.

Table 2.2: Relative differences between replicate values for 3 field samples containing all 4 analytes

		Chlorpyrifos		Endosulfan-I		Endosulfan-II		Endosulfan Sulfate	
Sample	**Run**	**Conc.[b]**	**Differences[c]**	**Conc.[b]**	**Differences**	**Conc.[b]**	**Differences**	**Conc.[b]**	**Differences**
Name		Cs^o (ug/l)	(%)	Cs^o (ug/l)	(%)	Cs^o (ug/l)	(%)	Cs^o (ug/l)	(%)
Bdr	A[a]	5.56	1.9	4.95	4.7	1.50	5.3	5.89	3.2
Bdr	B	6.01		5.98		1.85		6.71	
Dd	A	1.52	1.6	1.30	0.3	0.99	10.5	1.95	0.5
Dd	B	1.62		1.32		0.64		1.99	
G	A	0.24	2.1	0.20	4.3	0.13	3.6	0.19	7.6
G	B	0.27		0.24		0.11		0.25	

a: "A' and 'B' refer to the first and second values of a replicate pair.
b: Concentration, rounded to 2 significant figures.
c: Relative differences, ABSOLUTE(A-B)/(A+B)/2, as %.

Table 2.3: Differences between replicate values for mixed standards containing all 4 analytes

	Chorpyrifos	Endosulfan I	EndosulfanII	Endosulfan Sulfate
Sample	**$\lvert A- B^a \rvert$ (pg)**	**$\lvert A - B \rvert$ (pg)**	**$\lvert A - B \rvert$ (pg)**	**$\lvert A - B \rvert$ (pg)**
Standard	1.69	7.18	6.65	5.37
Standard	2.66	6.03	3.45	0.23
Standard	0.5	2.04	1.59	0.17
Standard	0.02	0.54	0.09	0.44
Standard	0.18	1.14	0.27	0.06
Standard	0.21	1.1	0.15	0.79
Standard	0.15	0.75	2.46	0.47
Standard	0.01	0.29	0.31	0.07
Standard	0.22	0.12	0.57	1.31
Standard	1.51	13.04	0.45	0.87
Standard	2.85	28.49	0.89	8.7
Standard	1.76	20.42	0.16	3.19
Standard	2.8	63.47	0.25	4.89
Standard	0.27	52.14	0.41	3.22
Standard	0.56	1.58	1.26	0.51
Standard	0.55	0.32	2.77	1.9
Standard	0.8	1.82	4.51	0.31
Mean differences	**0.98**	**11.79**	**1.54**	**1.91**
Standard Deviation of differences	**1.0**	**19**	**1.9**	**2.4**

a: 'A' is the first and 'B' is the second measured value of a pair of replicates, the differences are the absolute differences ($\lvert\ \rvert$).

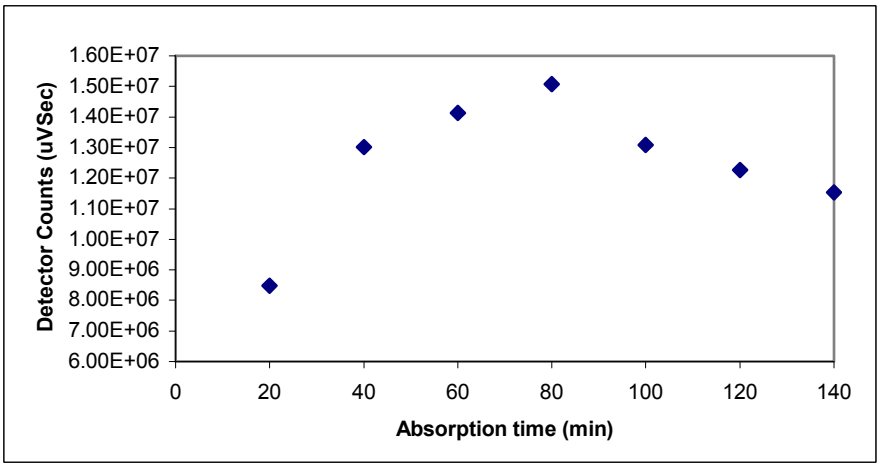
Note that Table 2.3 includes standards with concentrations ranging from 0.1µg/L to 5.0µg/L. The mass of analyte absorbed by an SPME fibre is approximately linear with concentration; for a 1µg/L mixed analyte sample, the mass absorbed is in the range of 200 to 400pg.

2.4.4 EQUILIBRIUM RUNS

The reproducibility of analyses depends primarily on reproducing extraction conditions - stirrer speed, temperature of the sample during extraction, position of the fibre rather than extracting for long enough to approach the equilibrium partitioning of the analyte between the fibre and the sample. Nonetheless, an experiment was conducted to estimate the time required to approach equilibrium.

The tests were done at 20°C. The SPME fibre fully exposed (10mm) to a mixed standard, containing all 4 analytes, of concentration 1µg/L (ppb) for a range of absorption times - 20 minutes to 140 minutes. The mixed standard in glass vial was stirred at maximum (N = 625rpm), using the magnetic stirrer and Teflon coated stirrer bar set-up. The results for endosulfan sulfate Standard at the 1µg/L level are shown in Figure 2.8.

Figure 2.8: Graph of detector counts versus absorption time for 1µg/L endosulfan sulfate



Maximum counts (directly proportional to mass absorbed) were reached at about 80 minutes. Thereafter, an apparent decrease in concentration (mass absorbed) occurred. All the analytes yielded similar results.

2.4.5 DETERMINATION OF THE METHOD DETECTION LIMIT

There are several approaches to defining and determining the Method Detection Limit. A widely accepted definition is based on using low concentration spikes into a blank matrix and calculating the standard deviation of 7 to 10 determinations. The Method Detection Limit (MDL) is then defined as 3 times the standard deviation obtained for the analyte concentration, for a concentration not higher than 10 times the MDL. This definition is similar to that used by the US EPA.

Thus: $$\mathrm{MDL} = \frac{3 * (\text{Standard deviation}) * (\text{Concentration in Solution [Cs]})}{\text{Mean Peak Area}}$$

A series of experimental runs was conducted in order to determine the Method Detection Limit of SPME sampling and analysis. Initially, the MDL was unknown hence the value of 10 times the MDL was unknown. Three different (successively lower) concentration levels were therefore selected (i.e. 1.00µg/L, 0.04µg/L and 0.02µg/L) to determine the MDL, and seven repeat runs were performed at each concentration. The standard deviation was then determined for the seven runs at each concentration level. The results are reflected in Table 2.4.

Table 2.4: Table of MDL values for 3 mixed standards at 3 different concentration levels

	Chlorpyrifos			Endosulfan-alpha			Endosulfan-beta			Endosulfan Sulfate		
Conc. (ug/l)	Std. Dev.[a]	Mean Peak Area	3*Std. Dev. ug/l	Std Dev.	Mean Peak Area	3*Std. Dev. ug/l	Std Dev.	Mean Peak Area	3*Std. Dev. ug/l	Std Dev.	Mean Peak Area	3*Std. Dev. ug/l
0.02	1780	47209	0.002	44251	111505	0.024	10299	72745	0.008	3680	78294	0.003
0.04	5482	76207	0.009	8726	135822	0.008	19315	123762	0.019	13139	102209	0.015
0.1	25573	360256	0.021	37406	831822	0.013	34274	639102	0.016	20966	447572	0.014
		MDL	0.010		MDL	0.016		MDL	0.014		MDL	0.009

a) Standard deviation

The MDL was estimated as the average of the 3*standard deviation figures for 0.02 and 0.04 µg/L.

2.5 DISCUSSION

Vigorous stirring (agitation) improves reproducibility. The reproducibility at absorption times of 20 and 25 minutes improved significantly over 5, 10 and 15 minutes (Figures 2.7 and 2.8). The improvement in reproducibility between 20 and 25 minutes did not appear to be significant. Therefore a standard absorption time of 25 minutes was chosen. This time is also approximately equal to the GC cycle time (thermal desorption and elutriation), enabling optimum productivity of analyses to be achieved.

Figure 2.7 displays the results of a stirred chlorpyrifos standard against the results of an unstirred chlorpyrifos standard of the same concentration (1µg/L). Runs for the stirred and unstirred standards were conducted over the same range of absorption times in each case. In each situation, replicate runs were performed. It is clear that the unstirred runs produced inconsistent, erratic and irreproducible results, particularly for absorption times of up to 15 minutes. On the other hand, the replicate results for the stirred standard rendered reproducible results. This result is consistent with the use of agitation (magnetic stirrer or sonication) as a standard procedure, as reflected in the literature.

For the 3 field samples analysed using the SPME method, the relative differences (difference/ average value, as %) between replicate analyses ranged from 1 to 10%, for analyte concentrations in the range 0.1 to 6.0 µg/L. Average differences between replicates of standards analysed ranged from 1 to 12 pg for standards with concentration in the range 0.1 to 5µg/L (mass absorbed approximately 20 to 800pg).

The MDL for the SPME method was found to be 0.010µg/L, for chlorpyrifos, 0.016µg/L for endosulfan-alpha, 0.014µg/L for endosulfan-beta and 0.009µg/L for endosulfan sulfate. Compared to other techniques of analysis, such as Solid Phase Extraction (SPE) and Enzyme Linked Immunoassys (ELISA), the SPME method rendered consistently good sensitivity and reproducibility without being time-consuming or labour-intensive.

2.6 CONCLUSIONS

Vigorous stirring (agitation) of the sample is essential in order to achieve good reproducible results.

For the analytes under consideration, an absorption time of 25 minutes was demonstrated to be sufficient to yield reproducible results.

Under the optimum agitation (N = 625rpm) conditions and the absorption time (25 minutes) established for the analytes under consideration, the SPME method yielded excellent reproducibility (average differences between replicate standard analyses ranged from 1 to 12 pg for standards with concentration in the range 0.1 to 5µg/L (mass absorbed approximately 20 to 800pg) over the experimental range of concentrations. For the 3 field samples analysed, the relative differences between replicates ranged between 1 and 10% for the 4 analytes under consideration, for analyte concentrations in the range 0.1 to 6.0 µg/L.

The MDL is excellent at 0.009µg/L to 0.016µg/L for the 4 analytes studied. SPME sampling is a very good technical, as well as practical method. It is also cost-effective. There is no sample preparation involved as for SPE, and therefore SPME reduces the time required for analyzing for pesticides in a sample, and produces reproducible and reliable results.

SPME sampling yields a lower detection limit than SPE, an added advantage.

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Chapter 3:

THE DEVELOPMENT OF A METHOD FOR OBTAINING AN INTEGRATED (TIME WEIGHTED AVERAGE) SAMPLE OF A PESTICIDE CONTAMINATED STREAM USING A SOLID PHASE MICROEXTRACTION (SPME) DEVICE

CONTENTS

3.1 INTRODUCTION

Pesticide contaminant concentrations in surface streams may exhibit marked temporal variation, with time scales ranging from hours to months to that of an agricultural season. Temporal variations in concentration may relate to factors such as spraying and irrigation patterns, rainfall, soil characteristics, local meteorology and occasional spills of the pesticides during mixing. A great deal of data exists demonstrating the daily and seasonal variation[1], but little data is available on the hourly or diurnal variation of contaminant levels. Comparatively infrequent (typically weekly) grab samples may significantly over- or underestimate average concentrations.

An accurate assessment of the water quality in a particular region currently requires a relatively frequent (and costly) sampling regime. The costs of attempting to assess water quality at a number of sites through frequent grab sampling are prohibitive. In practice, grab sampling at intervals varying from twice per week through to weekly or at greater time intervals is practiced, and average values are inferred from these relatively infrequent samples. For example, the large scale (46 pesticides, 58 sampling sites over 3 years, 2 200 samples) US Geological Survey assessment of the status and trends in the quality of the United States' surface and ground water resources estimated the Time-Weighted Mean concentrations by associating each sample with the time interval halfway to the date of the preceding sample and halfway to the date of the succeeding sample. A sampling method that yields a Time Weighted Average (TWA) concentration over a comparatively extended period (24 hours or longer) using a single sample would give a more accurate picture of prevailing contaminant levels. If the cost of the TWA sampling system is low by comparison with frequent grab sampling or the use of an autosampler (automated grab sampling over 24 hours), the method would not only yield better data but would have cost advantages as well. Lowering the cost of sampling and analysis, and more representative (with respect to time-variation) data would enable the implementation of a better water quality assessment, monitoring and management system. One of the specific objectives of this research project was thus to evaluate the utility of solid phase microextraction fibres and a programmable sampler as tools to achieve integrated sampling of water resources being tested for pesticide contamination.

It should be noted that an estimate of contaminant load – the mass flow of contaminants during a specified period - in these streams requires a simultaneous estimate of stream flow rates as well as measurements of contaminant concentrations.

The predominant application of SPME sampling uses the characteristic that, when an SPME fibre is exposed to a sample with analyte concentration C_s, for sufficient time for equilibrium to be reached, the analyte partitions between the sample and the fibre coating in accordance with:

$$C_f = K_{fs} * C_s, \qquad \text{…….. (1)}$$

where K_{fs} is the distribution constant.

The concentration of the analyte in the fibre, C_f, is defined by:

$$C_f = m_f / V_f \qquad \text{…….. (2)}$$

The mass of the analyte on the fibre, m_f, is determined by Gas Chromatography (GC), and hence the fibre concentration and the sample concentration C_s may be calculated using the known fibre volume V_f and Equations 1 and 2. However, the mass loading rate of analyte into the fibre decreases with time, and approaches zero asymptotically as equilibrium is approached. Thus true equilibrium is only reached at 'infinite time'.

If the K_{fs} value is accurately known, and equilibrium is approached to within, say, 5% the sample concentration may then be calculated as follows:

$$C_s = (1/K_{fs}).C_f = (1/K_{fs}).(m_f/V_f). \quad \ldots\ldots\ldots (3)$$

In practice, the Gas Chromatograph response (Detector Counts) for the unknown sample is compared to a calibration curve prepared by exposing the fibre to analytical standards of known concentration, under identical exposure conditions and for the same time period as for the unknown sample. The most important variables determining exposure conditions are absorption time, stirrer speed, sample temperature and position in the sampling vial.

In contrast, the use of an SPME fibre for the determination of the TWA concentration over a given period is based on sampling under non-equilibrium conditions. Under conditions sufficiently far from equilibrium, the mass-loading rate of analyte into the fibre coating is approximately linear with time and concentration. This unsteady state behaviour of the system, within suitable constraints, may therefore be used to obtain a TWA sample of analyte concentrations. In addition to the mass loading rate over the sampling period, the retention of the analyte absorbed during sampling and sensitivity to low concentrations are important additional criteria for a quantitative estimate of TWA concentrations.

One of the objectives of this project was the development of a method to obtain an integrated (TWA) sample, using SPME. The sampling time period was not specified. The data collected during the course of the project indicated that significant variations in the concentrations of pesticides occur over relatively short periods of time – essentially from hour to hour. A sampling system yielding a 24-hour TWA sample would eliminate uncertainty as to the representivity, with respect to the 24-hour period, of a daily grab sample. A longer term, but more difficult, objective would be to obtain a sample representative of the TWA concentration over a 7-day period, and the simultaneous estimate of the peak concentration that may have occurred during the same period.

3.2. THEORETICAL CONSIDERATIONS

3.2.1 PRIOR WORK USING SPME TO DETERMINE THE TWA CONCENTRATION OF AIR CONTAMINANTS, BASED ON THE STEADY STATE ASSUMPTION

Thus far attempts to use SPME to obtain a TWA sample have focused on the analysis of volatile or semi-volatile compounds (e.g. hydrocarbon mixtures) in air,[2,3,4] based on the following theoretical approach.

Consider a stream with time varying pollutant concentration, C(t). The Time Weighted Average Concentration C_{TWA} over a total time period t_T, for a medium with time varying concentration C(t), may be defined as:

$$C_{TWA} = \frac{1}{t_T}\int_0^{t_T} C(t)dt \quad \ldots\ldots\ldots (4)$$

Continuous measurements of concentration are generally not available, and the TWA concentration is calculated by taking a number of grab samples (say n samples), with measured concentrations C_i, at time intervals t_i. The time intervals are not necessarily equal, and each sample is assumed to be representative of the interval between samples. The TWA Concentration is then approximated by:

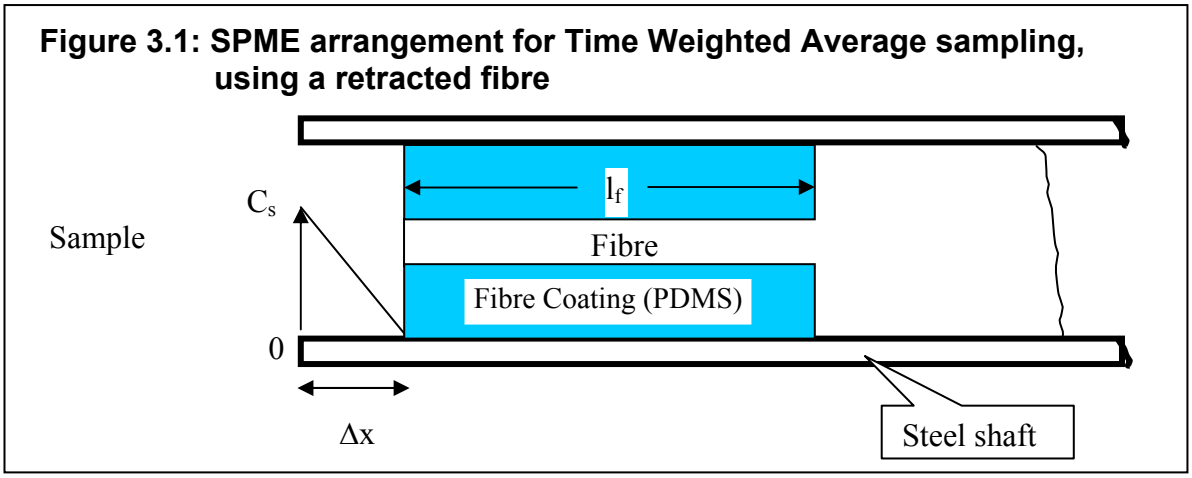
$$C_{TWA} = \frac{\sum_{i=1}^{n} C_i t_i}{\sum_{i} t_i} \qquad \ldots\ldots\ldots (5)$$

The total time over the sampling period is $t_T = \Sigma t_i$.

Martos and Pawliszyn[2] (and others researchers[5]) demonstrated that the SPME fibre could be used as a passive (diffusive) sampler to obtain the TWA concentrations of volatile and semi-volatile compounds in air. In these applications, the device was operated in pseudo-steady state mode and the steady state assumption of sampling rate was made to estimate the TWA concentration.

This approach used the arrangement shown in Figure 3.1.

Figure 3.1: SPME arrangement for Time Weighted Average sampling, using a retracted fibre



The concentration at the face of the steel shaft was assumed to equal the free stream concentration $C_{s\infty}$ (the 'free stream concentration' is the concentration far from the influence of the fibre, at an 'infinite' distance from the fibre) and the concentration was assumed to be approximately zero at the fibre-sample interface. The fibre, length l_f , was retracted a distance Δx into the steel shaft. Under these pseudo steady state conditions, Fick's First Law was assumed to be valid. The conditions are pseudo steady state in the sense that the concentration at the interface is clearly increasing with time, but the time dependent change is much slower than that which occurs immediately after the fibre is exposed to the sample.

Hence, for analyte diffusivity in the sample D_s, constant (mass) concentration gradient dC/dx and (mass) flux **j**, Fick's First Law is:

$$j = -D_s \frac{dC}{dx} \qquad \ldots\ldots\ldots (6)$$

The flux **j** has units of mass/(time*area).

For constant cross-sectional area A,

$$j = \frac{1}{A}\frac{dm}{dt} = -D_s \frac{dC}{dx} \qquad \ldots\ldots\ldots (7)$$

The rate of mass transfer to the fibre is implicitly controlled by diffusion in the sample phase. The finite diffusion path Δx may be fixed by withdrawing the fibre into the steel sheath and assuming that, for relatively short sampling times the concentration at the fibre –sample interface is approximately zero, hence the sampling rate or mass loading rate dm/dt, with units of mass/time, may be approximated by:

$$\frac{dm}{dt} = \frac{A.D_s}{\Delta x}(C_s - C_i) = R(C_s - C_i), \qquad \text{…….. (8)}$$

where R is the volumetric sampling rate, with units of volume/time.

If the interfacial concentration C_i = 0, the mass loading rate (mass sampling rate) is proportional to the sample concentration C_s, and is given by

$$\frac{dm}{dt} = \frac{A.D_s}{\Delta x}(C_s) = R.C_s. \qquad \text{…….. (9)}$$

In practice, the factor R, the volumetric sampling rate is determined empirically by measuring the mass loading rate for a known concentration C_s.

The total mass m_T absorbed onto the fibre to time t_T is thus given by:

$$m_T = R.\int_{t=0}^{tT} C_{s\infty}(t)dt \qquad \text{……. (10)}$$

The sampling rate is measured empirically by exposing the fibre to a standard solution or solutions, for increasing time periods. The total mass loaded onto the fibre, m_T, is determined by GC analysis. The GC calibration curve is prepared using direct injection of standards. Hence the mass m_T may be determined directly and the TWA, for a given exposure time $t = t_T$, may be calculated using equations 4 and 10:

$$C_{TWA} = m_T/(R.t_T)$$

Martos and Pawliszyn[2] recommended that the application of this analysis be restricted to a fibre loading of less than 5% of the equilibrium loading value. While this is consistent with the assumption that the concentration at the interface is approximately zero, it is clearly a significant restriction since it reduces the sensitivity of the method – the overall sensitivity of the method is directly related to the total mass loaded onto the fibre.

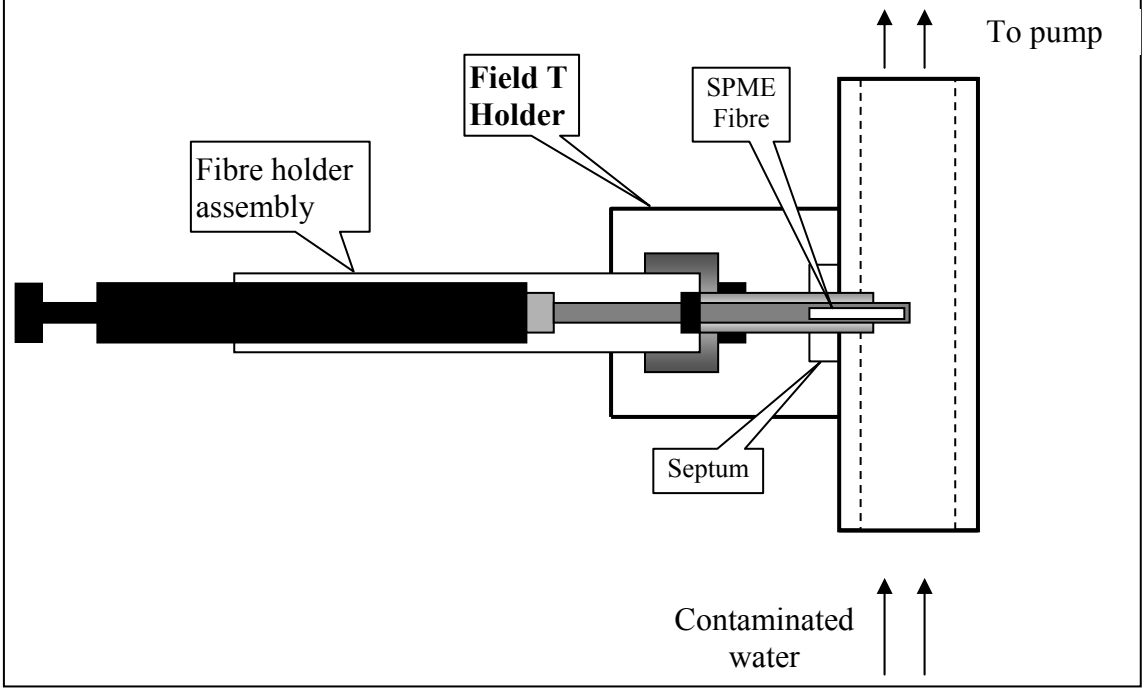
3.2.2 THE THEORETICAL BASIS FOR THE APPLICATION OF SPME TO OBTAIN A TWA CONCENTRATION OF PESTICIDES (ANALYTES) IN WATER

The application of SPME to the TWA sampling of pesticides in water has to contend with factors significantly different compared with the sampling of air pollutant concentrations. Diffusivities in water are two to 4 orders of magnitude lower than diffusivities in air[6]; analyte concentrations may be two to 3 orders of magnitude lower, of the order of 0.1 to 10 ppb compared with several hundred ppb, as well. A theoretical framework that restricts the sampling method to 5% of the equilibrium fibre concentration would not produce the required sensitivity at low contaminant concentrations. A more detailed theoretical framework is required to determine feasible experimental or operating conditions, and the limitations of the method, for the application of SPME to TWA sampling.

The proposed experimental sampling device is similar to that used for air sampling, except that the fibre may be positioned flush with the entrance of the steel shaft, or retracted by distance Δx from the entrance of the steel shaft (see Figure 1).

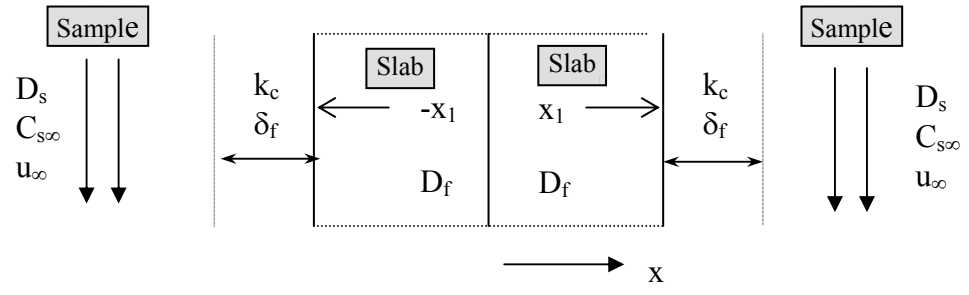
The fibre holder is located in a custom-made field sample 'T' holder that is designed to protect the fibre and to enable well defined velocity field to be created past the sampling device, as shown in Figure 3.2.

Figure 3.2: Schematic diagram of the field sampling device



TWA air sampling, the fibre holder with retracted fibre is simply exposed to the air being sampled. For TWA sampling in water, a defined flow field past the retracted fibre is required. (The need for a defined flow field past the steel sheath housing the fibre created by the flow induced by a pump (Figure 3.2) is clarified in the analysis below.) The theoretical analysis of the system, using Cartesian Co-ordinates, is based on the model depicted in Figure 3.3.

Figure 3.3: Theoretical model for the sampling system: unsteady diffusion into an infinite slab of thickness $2x_1$



Nomenclature:
Ds and D_f are the diffusivities of the analyte in the sample medium (water) and the fibre coating respectively, u_∞ is the velocity of the sample medium far from the surface of the face of the steel shaft, Cs_∞ is the sample analyte concentration far from the influence of the absorbing fibre, C_f is the analyte concentration in the fibre and x_1 the length of the fibre.

The theoretical model assumes that symmetrical diffusion occurs from both sides of the infinite plate. By symmetry, no mass transfer occurs across the centreline. This system models mass transfer into a fibre of length x_1, with no transfer beyond the end of the fibre. Mass transfer occurs from the sample across the film of fluid of thickness δ_f adjacent to the fibre face. The concentration boundary layer thickness, the distance to a point at which the concentration reaches 99% of the free stream sample concentration $C_{s\infty}$, is designated δ_c, and the film thickness is δ_f. The concentration gradient at $x = \delta_c$ is by definition negligible and thus no mass transfer occurs in the x direction across the concentration boundary layer. Molecular mass transfer in the direction of flow is assumed to be negligible by comparison with convective mass transfer (that is, mass transfer due to the bulk fluid motion) in the flow direction. In all the situations under consideration, the flow is well within the laminar flow regime. (Note that convective mass transfer may occur in laminar flow – it should not be assumed to be mass transfer due to turbulence in the flow.)

Fick's Second Law may be derived from an unsteady state mass balance in the *sample*, designated by subscript s:

$$\frac{\partial C_s}{\partial t} = D_s \frac{\partial^2 C_s}{\partial x^2} \qquad \text{..... (11)}$$

Similarly, a mass balance over a shell perpendicular to the x direction in the slab, the analogue for the fibre coating, yields:

$$\frac{\partial C_f}{\partial t} = D_f \frac{\partial^2 C_f}{\partial x^2}. \qquad \text{..... (12)}$$

In Equation 12, the subscript f denotes the fibre coating, C_f is the concentration at any time t > = 0 and point x in the fibre, and D_f is the diffusivity of the analyte in the fibre. The subscripts s and f refer to the sample phase (typically air or water) and the fibre coating phase respectively; the subscript i refers to the interface and ∞ to 'infinity' with respect to distance from the fibre face.

Let C_s be the concentration in the sample, $C_{s\infty}$ the free stream concentration, C_{s0} the initial concentration of the fibre and the sample in the vicinity of the fibre (C_{s0} = 0), C_f the fibre concentration, C_{fCL} the fibre concentration at the model centreline corresponding to the end of the fibre and C_{fi} the concentration at the interface between the fibre and the sample, on the fibre side.

At the interface: $C_{fi} = K_{fs}*C_{si}$. As before, K_{fs} is the distribution constant.

The following dimensionless variables may then be defined[7]:

Dimensionless concentrations: $Cs^* = \frac{Cs_\infty - Cs}{Cs_\infty - Cs_0}$ and $C_f^* = \frac{K_{fs}.Cs\infty - C_f}{K_{fs}.Cs_\infty - C_{f0}}$. ..... (13)

Dimensionless distance: $x^* = x/x_1$ ……. (14)

and dimensionless time: $t_f^* = (D_f t/x_1^2)$ or $t_s^* = (D_s t/\delta_f^2)$ ……. (15)

Equations 11 and 12, expressed in terms of these dimensionless variables, become the dimensionless form of Fick's Second Law::

$$\frac{\partial C^*}{\partial t^*} = \frac{\partial^2 C^*}{\partial x^{*2}} \quad \text{……. (16)}$$

Note that the dimensionless distance and time may equivalently be expressed in terms of any characteristic length other than x_1. The differential Equations 11 and 12, and the dimensionless equivalent Equation 16, are subject to the following conditions.

The initial condition is:

at t=0, $C_s = C_{s0}$, $C_f = C_{f0}$;

that is, at $t^* = 0$; $C_s^* = 1$ and $C_f^* = 1$. ……. (17)

(At $t = \infty$, $C_f = K_{fs}.C_{s\infty}$, therefore $C_f^*{}_\infty = 0$.)

For the fibre, the first boundary condition, BC1, is:

$$\frac{\partial C_f}{\partial x} = 0\Big|_{x=0} \text{ i.e., by symmetry.} \quad \text{..... (18)}$$

That is, the concentration gradient at the centreline is zero and no mass transfer occurs across the centreline.

Therefore this boundary condition, expressed in terms of dimensionless variables, becomes:

$$\frac{\partial C_f^*}{\partial x^*} = 0\Big|_{x^*=0}. \quad \text{……. (19)}$$

The second boundary condition, BC2, is based on a mass balance across the fibre-sample interface. At the interface, the mass leaving the sample is equal to the mass being absorbed by the fibre. Thus BC2 is derived from the following relationships.

The pseudo steady state mass flux equation describing mass transfer from the bulk of the fluid (at concentration $C_{s\infty}$) to the interface (at concentration C_{si}) is:

$$j_s = k_c [C_{s\infty} - C_{si}] ; \quad \text{……. (20)}$$

where $\mathbf{j}_s$ is the mass flux ***in the sample***, C_{si} the sample concentration at the interface and k_c is the mass transfer coefficient. Equation 20 is based on the concept that mass transfer is occurring across a film of fluid, of thickness δ_f. (See Figure 3.3.) The concentration boundary layer occurs, by definition, at the distance from the interface at which the concentration reaches 99% of the free stream concentration $C_{s\infty}$. The boundary layer thickness is not constant across the interface. A detailed analysis (given in section 3.3 of this chapter) of the boundary layer shows that the average film thickness, $\delta_f = (2/3)\delta_c$[8]. In laminar flow, $k_c = D_s/\delta_f$. Note that the flux **j** is the rate of mass flow per unit area.

The sample free stream concentration $C_{s\infty}$ is assumed to remain constant or to change slowly in relation to the overall response time of the system. For the situations under consideration, the interfacial concentration changes slowly compared with the initial transient response. This is the pseudo steady state assumption. Equation 20 thus expresses the mass flux from the sample fluid across the interface, under the specified conditions.

The mass flux $\mathbf{j_f}$ ***in the fibre*** may be described by Fick's First Law:

$$j_f = -D_f \frac{dC_f}{dx} \qquad \ldots\ldots. (21)$$

At the interface ($x=x_1$), the mass flux (mass transfer rate per unit area) across the interface and therefore the mass transfer rates must be equal but opposite in sign; i.e., $j_s = -j_f$. (In both cases, the mass transfer area is the interfacial area.)

Thus the second boundary condition, at the sample-fibre interface, is:

$$k_c[Cs\infty - Csi] = +D_f \frac{dC_f}{dx} \Big|_{x=x1} \qquad \ldots\ldots. (22)$$

At the interface, $x=x_1$ and $C_f = C_{fi} = K_{fs}.C_{si}$. The second boundary condition (BC2) then becomes:

$$\frac{dCs^*}{dx^*} = -\frac{k_c.x_1}{K_{fs}.D_f} Cs^*(1,t^*) = -Bi_{MT}.Cs^*(1,t^*) \qquad \ldots\ldots. (23)$$

The rationale for the use of the term Bi_{MT} in Equation 23 is as follows.

In laminar flow, $k_c = \frac{D_s}{\delta_f}$. Therefore, in Equation 23, we may define the coefficient of Cs* by:

$$\frac{k_c.x_1}{K_{fs}.D_f} = \left(\frac{D_s}{D_f}\right)\left(\frac{x_1}{\delta_f}\right)\left(\frac{1}{K_{fs}}\right). \qquad \ldots\ldots. (24)$$

The term on the left hand side of equation 24 may be interpreted as the ratio of the mass transfer resistance in the fibre, $R_{fibre} = \frac{x_1}{D_f.K_{fs}}$, to that in the film, $R_{film} = \frac{\delta_f}{D_s}$. That is, $\frac{k_c.x_1}{K_{fs}.D_f} = \frac{R_{fibre}}{R_{film}}$. In heat transfer theory, in a similar situation of the heat transfer to or from a solid to a fluid, the ratio of conductive heat transfer resistance (in the solid) to convective heat transfer resistance is termed the Biot Number (Bi).

In the transfer of heat to or from a conducting solid to a flowing fluid, the Biot Number is defined by $Bi = hL_c/k$, where h is the heat transfer coefficient, L_c a characteristic length and k the thermal conductivity.

By analogy, we therefore propose that the ratio of fibre-to-film mass transfer resistances be called the ***mass transfer Biot Number*** (**Bi_{MT}**). That is,

$$\frac{k_c.x_1}{K_{fS}.D_f} = \frac{R_{fibre}}{R_{film}} = Bi_{MT} .$$

Note that, in the initial transient period (of the order of seconds), the system behaves as if it is far from its boundaries and the (mass transfer) Biot number during this period is given by:

$$Bi_{MT} = \left(\frac{D_s}{D_f}\right)\left(\frac{1}{K_{fS}}\right) \qquad \text{……. (25)}$$

The dimensionless Equation 16, with the corresponding initial condition (Equation 17) and the boundary conditions (Equations 19 and 23) is identical to the corresponding heat transfer equation. The dimensionless time t* corresponds to the Fourier Number F0. Thus the solutions to this set of equations (Equations 16, subject to 17, 19 and 23) are identical to the corresponding solutions to the heat transfer equations.[9]

If the Biot number <<1 or >> 1, lumped parameter approximations may be used. In the mass transfer situation under consideration, Bi_{MT} <<1 (say <0.1) infers that the resistance to mass transfer in the fibre is negligible compared to that in the sample fluid. For Bi_{MT} >>1 (say Bi_{MT} >10), the inference is that the resistance to mass transfer in the sample fluid is negligible compared with resistance in the fibre. In each of these special cases, simplified expressions for the concentration profiles and the mass transfer rate in the system may be obtained, as given, for example, by Pawliszen for the case of mass transfer to a cylindrical fibre placed perpendicular to the flow field.[10]

The mass transfer Biot Number is important for the estimation of the linearity of the response of SPME fibre over a given time period. Thus, to define the necessary conditions for linearity with respect to time, the generally applicable (for all Biot numbers) exact solutions to the problem are required.

One solution to Equation 16 and the associated initial condition and boundary conditions is in the form of an infinite series involving sine and cosine functions. The dimensionless concentration profiles given by this series are[11]:

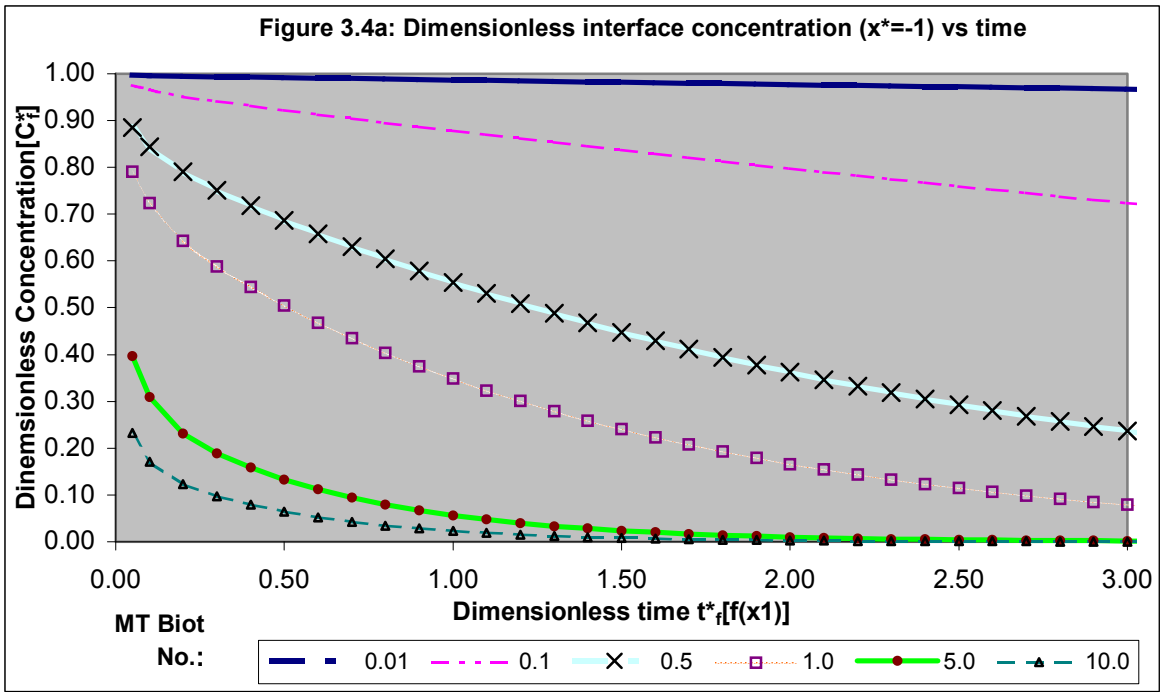
$$C^* = \sum_{n=1}^{\infty} C_n.\exp[(-\xi_n^2.t^*)(\cos(\xi_n.x^*))] \qquad \text{……. (26)}$$

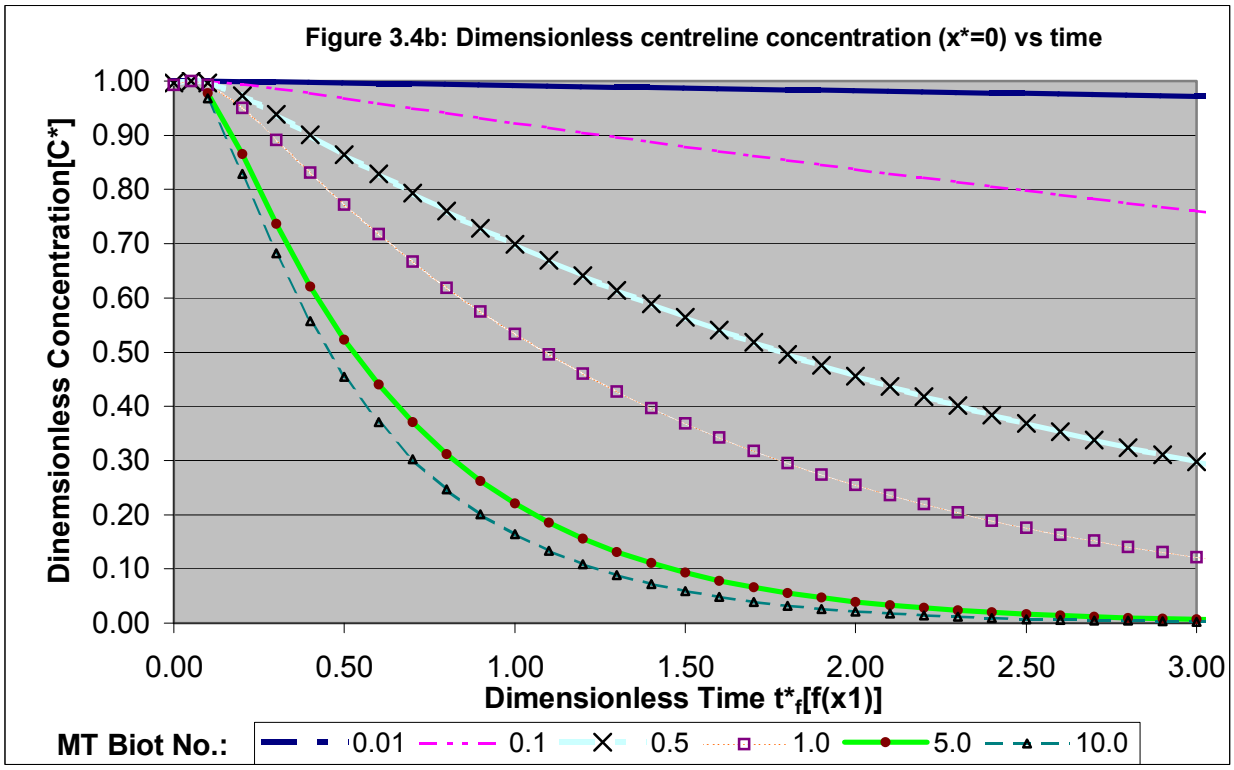
$$\text{where} \quad C_n = \frac{4.\sin\xi_n}{2.\xi_n + \sin(2.\xi_n)} \qquad \text{……. (27)}$$

$$\text{and } \xi_n \text{ is given by} \quad \xi_n.\tan(\xi_n) = Bi \text{, n=1 .. } \infty. \qquad \text{……. (28)}$$

Note that the concentration at the interface, C^*_i, occurs at x_1^*=1, and the concentration at the centreline occurs at x_1^*=0.

Figures 3.4a and 3.4b display plots of concentration vs time at the interface and at the centreline, using a 4 term (n=1 to 4) approximation to the infinite series represented by Equations 26 to 28.





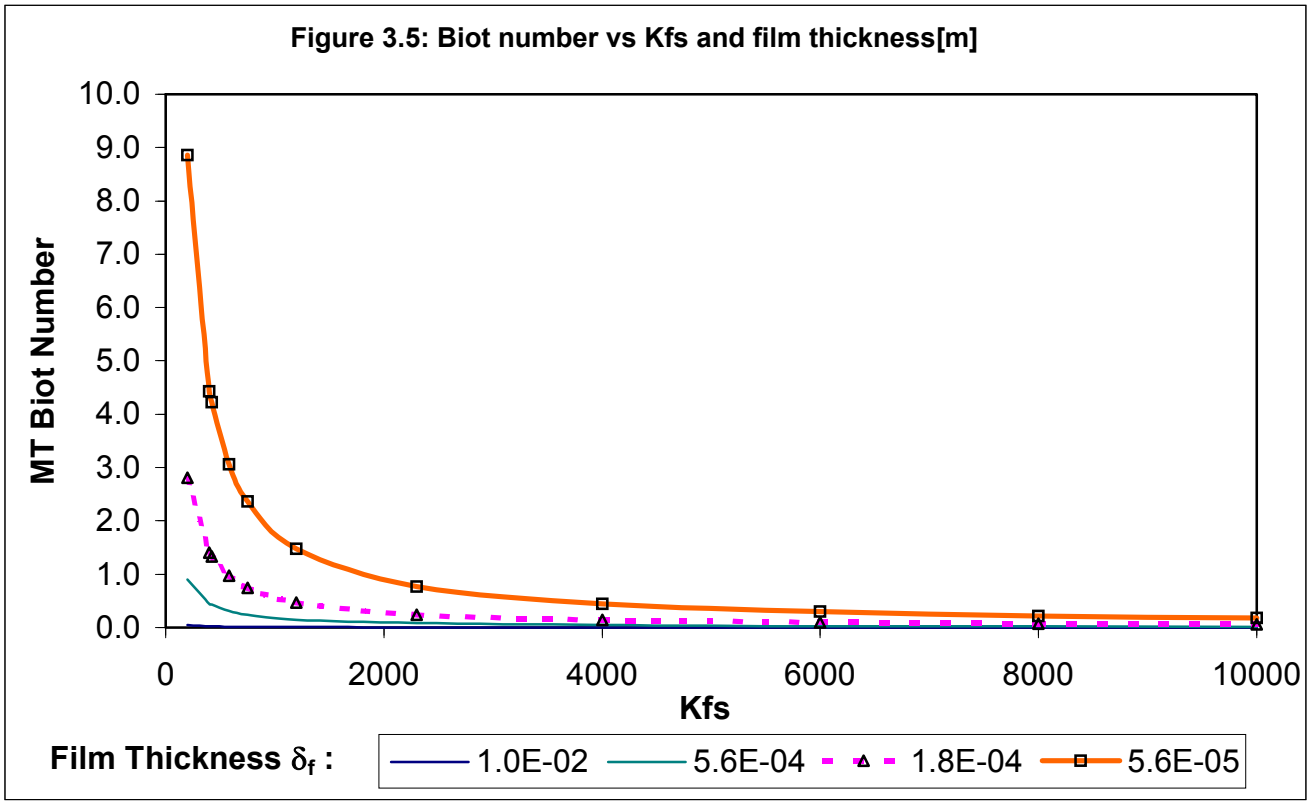
(MT: Mass Transfer)

The second boundary condition implies that the free stream concentration of the sample, $C_{s\infty}$, remains constant throughout the sampling period. A theoretical analysis that allows $C_{s\infty}$ to vary with time, a situation representative of real-time environmental sampling, is possible but would introduce considerable additional complexity into the theoretical solution. Instead of a rigorous theoretical analysis including a time-varying $C_{s\infty}$, the underlying assumption was made that the sampling system behaves as if it is exposed to a series of discrete exposures $C_{s\infty,i}$ for time intervals t_i, and that the mass transferred to the fibre during each of

these exposures is additive. The validity and limitations of this assumption must be tested experimentally. The assumption is in any case not valid if the concentration $C_{s\infty,i}$ drops below the interfacial concentration. This limitation will be discussed in more detail in the Conclusions.

For given values of D_s, D_f and x_1, the Biot number may be plotted against the distribution constant K_{fs} and film thickness δ_f as in Figure 3.5.

Figure 3.5: Biot number vs Kfs and film thickness[m]



The range of film thickness values used in Figure 3.5 encompasses different sample flow velocities past the fibre.

The ratio of mass transferred at time t or t* (m) to the equilibrium mass transferred, at 'infinite time', (m_∞) is given by:

$$\frac{m(t^*)}{m_\infty} = 1 - \sum_{n=1}^{\infty} [C_n . \exp(\xi_n^2 . t^*)] . \frac{\sin \xi_n}{\xi_n} \qquad \ldots\ldots (29)$$

where the constants C_n and ξ_n are functions of the Biot number.

This analysis is not necessarily restricted by the approximation that $C_i \sim 0$, or $C_i < 5\%$ of $C_{s\infty}$. Thus Equation 8 rather than the approximation given by Equation 9 must be used to estimate the total mass transferred to the fibre over the sampling period.

The sampling rate is given by:

$$\frac{dm}{dt} = \frac{A.D_s}{\Delta x}\left(C_s - C_i\right) = R.(Cs - Ci) \qquad \text{…….. (8)}$$

The total mass loaded into the fibre is then given by:

$$m_T = R.\int_{t=0}^{t_T} [C_{s\infty}(t) - C_i(t)]dt \qquad \text{……. (30)}$$

Since the concentration difference (the mass transfer driving force) is assumed to be approximately linear with respect to time for the conditions under consideration, an average volumetric sampling rate R_{ave} over the sampling period may be approximated by the relationships:

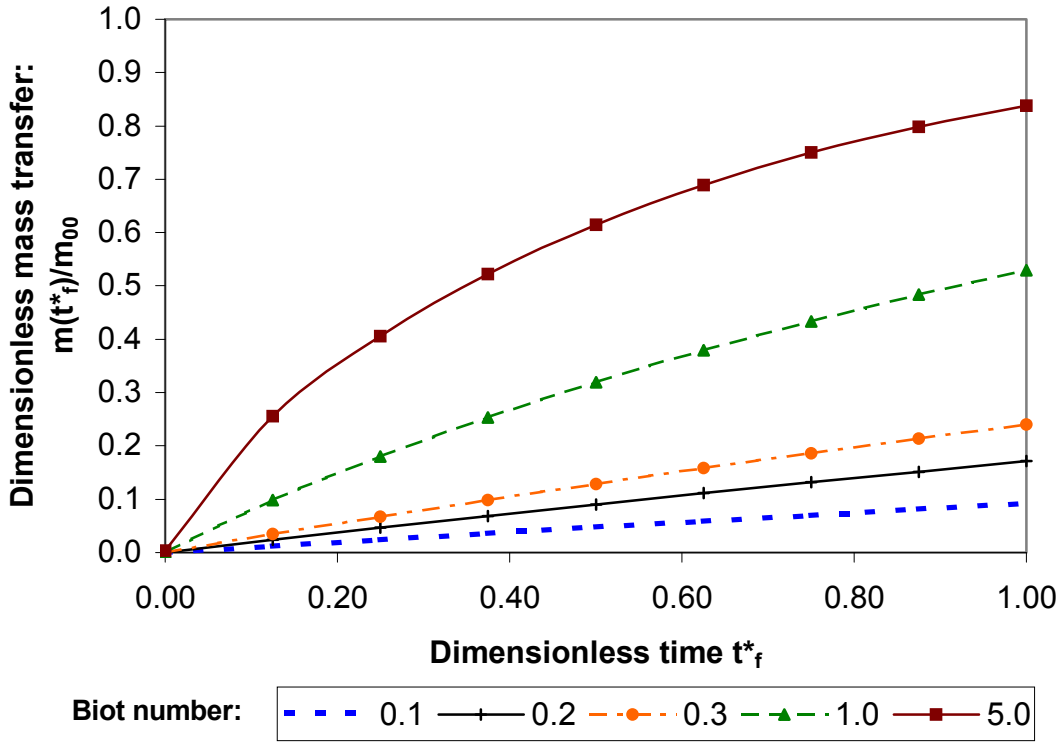
$$m_T = R_{ave}.\int_{t=0}^{t_T} C_{s\infty}(t)dt \; ; \qquad \text{……. (31)}$$

The average volumetric sampling rate is the arithmetic average over the time period, $R_{ave} = 0.5(R_0+R_{tT})$. As before, m_T may be measured using gas chromatography, R_{ave} may be determined empirically and the TWA concentration may be calculated using the relationship derived from Equations 4 and 31:

$$C_{TWA} = m_T/(R_{ave}.t_T).$$

Equation 29 may be plotted (Figure 3.6) in order to ascertain the range of linearity of the mass absorbed into the fibre with respect to time, for a range of Biot numbers. Figure 6 was plotted using a 4-term (n=1 to 4) approximation of the infinite series, Equation 26. The rate of mass transferred to the fibre is clearly strongly influenced by the Biot number.

Figure 3.6: Mass transfer to fibre vs dimensionless time
Parameter: Biot Number[Bi=(D_w/D_f)*(x_1/δ_f)*($1/K_{fs}$)]



The analysis may be extended to determine the conditions required to obtain a TWA over any given period.

3.3 MATERIALS AND METHODS

In order to estimate the Biot Number (using Equation 23) applicable to the experimental arrangement values of the diffusivities D_s and D_f, the fibre length x_1, the film thickness δ_f and the distribution constants K_{fs} are required. Only the value of x_1 (10mm) is known. The values of D_s, D_f and δ_f were calculated and the K_{fs} values were measured, using the procedures outlined in Chapter 2.

3.3.1 CALCULATION OF DIFFUSIVITIES

The correlation of Wilke and Chang[12] was used to calculate the diffusivities of chlorpyrifos and endosulfan in water. The basic correlation is:

$$D_{AB} = \frac{7.4 * 10^{-8} (\Phi_B . M_B)^{0.5} . T}{\mu_B . V_A{}^{0.6}}, \qquad \ldots\ldots\ (32)$$

where D_{AB} is the diffusivity of substance B through A (water), ϕ_B the association parameter, M_B the molecular mass of solvent B, T the absolute temperature[K], μ_B the viscosity of B[cP] and V_A the molal volume of solute A at the normal boiling point[cm^3/g]. In this equation, the diffusivity is given in units of cm^2/s.

Reliable correlations for the estimation of diffusivities in the fibre polymer coating do not appear to be available. Pawliszen[13] indicates that the diffusivity of an analyte in PDMS is about 5 to 6 times lower than that in water. A ratio of 6:1 was used, i.e., $D_f = (1/6)*D_s$.

3.3.2 ASSESSMENT OF ANALYTE RETENTION ON THE FIBRE

In the field sampling setting, the sampling system is exposed to a fluctuating sample concentraion over a period up to 24 hours. The theoretical basis of the method assumes that the analyte absorbed by the fibre does not desorb during periods of low analyte concentration during the sampling period. That is, the estimate of the TWA concentration given by Equations 4 and 30 assumes 100% retention of the analyte during the sampling period.

A preliminary assessment of analyte retention was done using the fibre exposed by 3mm beyond the steel shaft. The fibre was exposed to a spiked analyte solution (1ug/l) for a period of 15 minutes, under vigorous stirring, then exposed to analyte-free water for periods of 30 minutes to 180 minutes.

3.3.3 LINEARITY OF MASS ABSORBED WITH RESPECT TO EXPOSURE TIME AND CONCENTRATION

The theoretical curves indicate that the system has to be operated at Biot values less than 0.3 (approximately) for linearity with respect to exposure time to be achieved over a 24-hour period.

The effective diffusion path length, the film thickness δ_f, of the pesticide in water, may be estimated using the following relationship derived from an analysis of laminar boundary layer flow over a flat plate:[14]

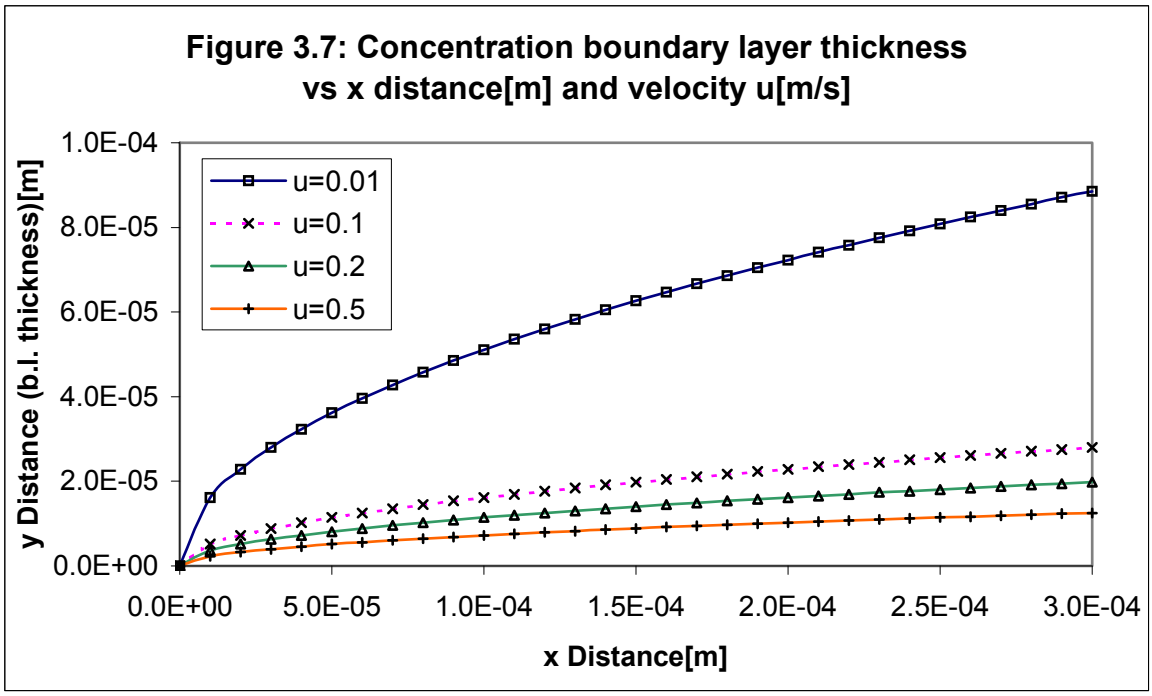
$$\frac{\delta c}{x} = 4.64\left(\frac{\mu}{x.u_\infty.\rho}\right)^{1/2}\left(\frac{D\rho}{\mu}\right)^{1/3} \qquad \ldots\ldots (33)$$

where δ_c is the concentration boundary layer, x is the distance from the leading edge of the fibre (stationary plate), u_∞ the velocity at infinity, μ the fluid viscosity, D the diffusivity and ρ the fluid density. Note that Equation 33 is of the form

$\frac{\delta c}{x} = 4.64\left(\frac{1}{Re}\right)^{1/2}\left(\frac{1}{Sc}\right)^{1/3}$, where Re is the Reynold's Number ($\frac{x.u_\infty.\rho}{\mu}$) and Sc is the Schmidt Number ($\frac{\mu}{D.\rho}$).

The (effective) film thickness δ_f is then given by: $\delta_f = (2/3)\square\delta_c$ ……. (34)

The concentration boundary layer thickness and hence the film thickness δ_f are thus functions of the parameters of Equation 33. The concentration boundary layer thickness varies with distance x from the leading edge of the fibre, and the free stream velocity u_∞ (the velocity far from the influence of the fibre). Figure 3.7 illustrates this functional relationship.



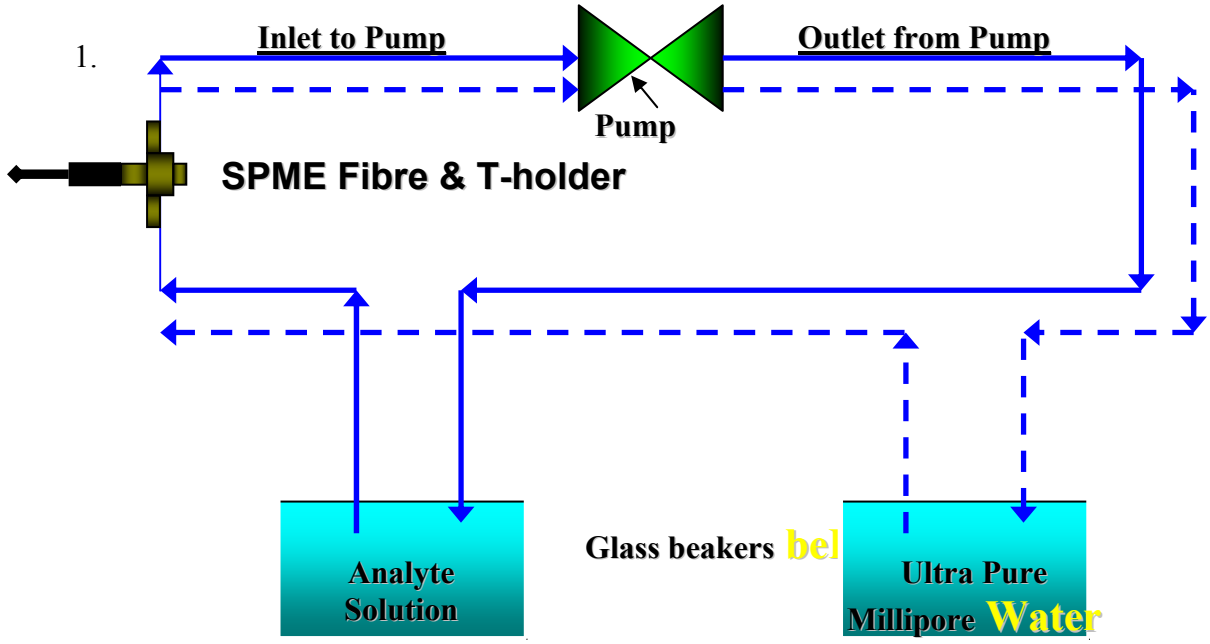
The theoretical analysis indicates that the required Biot Number could be achieved with the fibre mounted flush with the steel sheath for analyte-fibre systems with K_{fs} values above 10 000. However, for systems with lower K_{fs} values, the fibre should be retracted by about 0.5mm. The exact and reproducible positioning of the fibre in the 0.5mm retracted location required an adaptation of the fibre holder. On the other hand, positioning of the fibre flush with the steel shaft could be accomplished easily, with the aid of a low powered microscope. Thus the first linearity experiment was conducted with the fibre mounted flush with the steel shaft, for exposure (absorption) periods of up to 6 hours. The fibre was exposed to 1ug/l standard solutions for periods of 1, 2, 4 and 6 hours, to ascertain if the mass absorbed increased linearly with time over this period. A second set of experiments was planned with the fibre retracted 0.5mm, with exposure periods of up to 24 hours, but could not be carried out due to lack of time to modify the fibre holder.

The linearity of the GC detector response to concentration over the range of concentrations of interest was demonstrated with a set of direct injection runs.

3.3.4 EXPOSURE TO DIFFERENT CONCENTRATION PROFILES

The procedure used to test the assumption that exposure to different concentration profiles with the same theoretical TWA would yield the same (within experimental error) mass absorbed into the fibre was tested using a procedure based on that reported by Martos and Pawliszyn[15]. The fibre was positioned flush with the steel shaft, in a custom-made fibre holder and exposed to two different concentration profiles – the first consisting of an alternating series of 30 minute exposures to 1.0ug/l chlorpyrifos spiked into Millipore water, the second consisting of exposure to the 1.0ug/l solution continuously for 1h30m, then to the pure water only for the remainder of the period. In each case the mass absorbed into the fibre was ascertained by comparison against a direct injection calibration curve; the masses obtained for the two profiles were compared to each other.

Figure 3.8: System for assessing effect of exposure to different concentration profiles



Exposure to the solution or the pure water was achieved by changing the suction line (Figure 3.8) from the one beaker to the other at the required time. The pumping rate was constant throughout the period. The constant flow rate and the location of the fibre in the T-holder ensured a constant velocity (0.1 m/s) of the sample stream in relation to the face of the fibre.

In the first exposure pattern, the fibre system was exposed to the 1ug/l analyte solution and the pure water alternately for periods of 30 minutes at a time, for a total exposure-to-analyte time of 90 minutes over a 3-hour period. In the second exposure pattern, the fibre system was exposed to the analyte solution for a continuous period of 90 minutes, then to the pure water for 90 minutes. The theoretical TWA concentrations for the two exposure patterns were thus identical at 0.5ug/l. The mass absorbed into the fibre in each case was ascertained using GC.

The exposure profiles used are shown schematically in Figures 3.9a and 3.9b.

Figure 3.9a: First exposure pattern

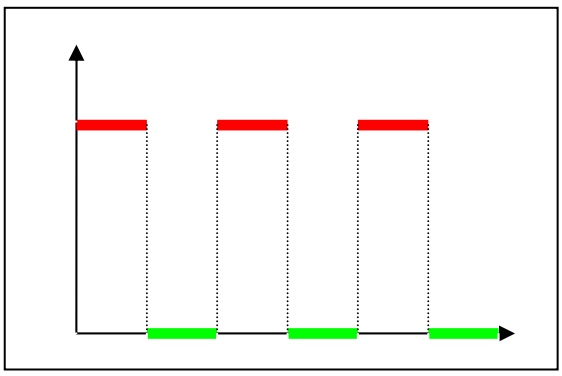
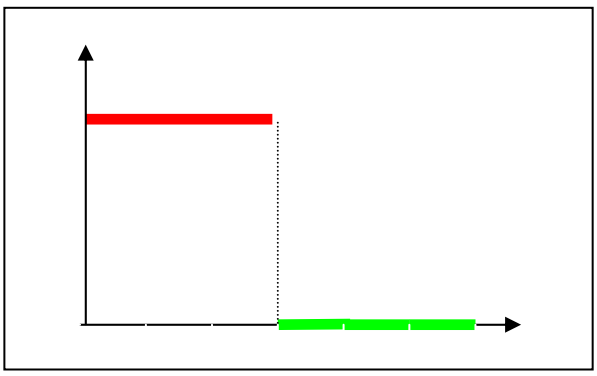


Figure 3.9b: Second exposure pattern

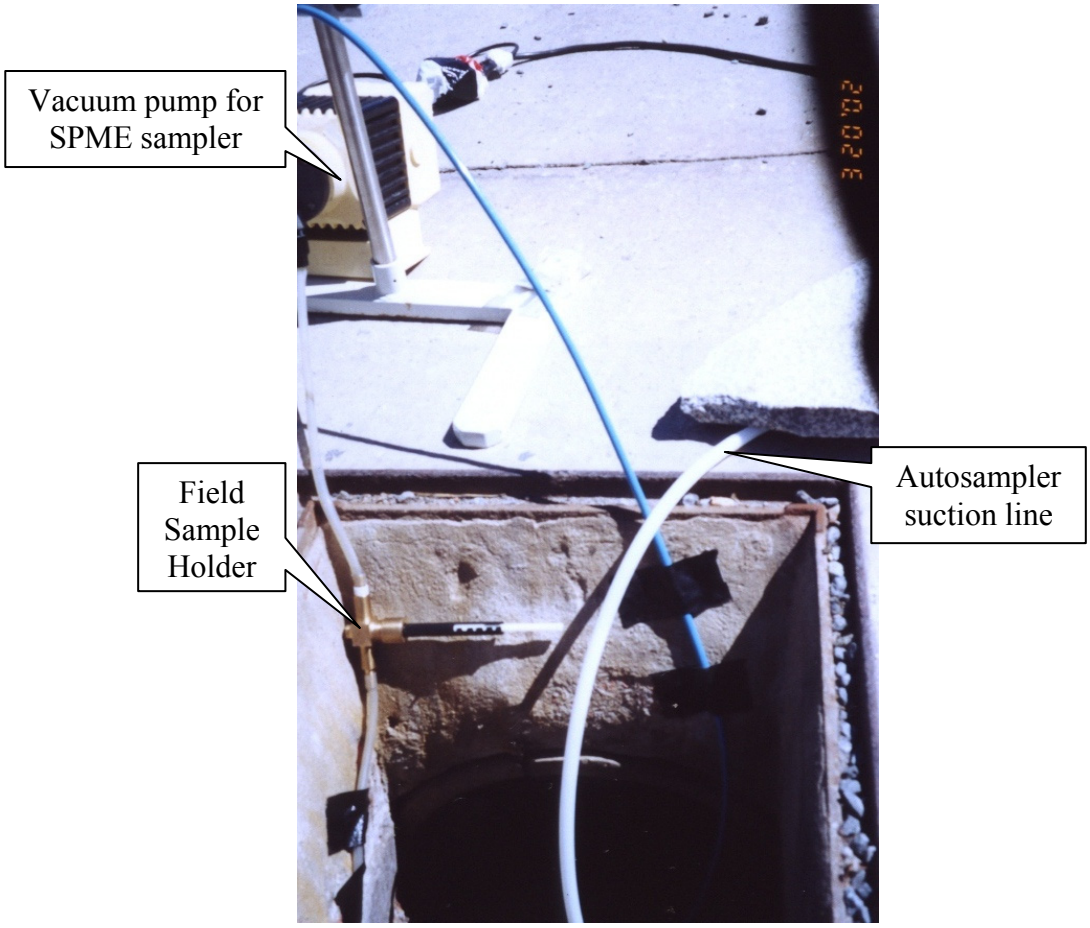


3.3.5 COMPARISON BETWEEN 24 X 1-HOUR GRAB SAMPLES AND THE 24 HOUR TWA SAMPLE

Field testing of the TWA sampling system was done at sites Bdr. At each site the Autosampler (Model: Buhler Montec XIAN 1000) was located close to the stream being sampled, and the Teflon suction line was positioned in the stream to allow the vacuum pump to sample the stream. The arrangement is shown in Figures 3.10a and 3.10b.

Figure 3.10a: Autosampler with suction line to drain BDr sampling point

Figure 3.10b: Field sampler holder, BDr sampling point



The autosampler was programmed to collect 24 x 1 litre samples, taken at hourly intervals, over a 24-hour period. The SPME fibre was positioned in the Field Fibre Holder as required for TWA sampling, mounted flush with the steel shaft. The TWA sampling system was exposed to the same stream by using a pumping circuit as shown in Figure 3.10, with the pump suction located close to the suction line of the autosampler. This arrangement thus allowed the two devices to sample essentially the same stream, over the same time period.

The 24-hourly samples were returned to the laboratory, refrigerated as normal, and were analysed using the standard SPME method.

The fibre holder was simultaneously returned to the laboratory for analysis. The mass of analyte absorbed into the fibre was established by comparison with a direct injection (external) calibration curve.

The single TWA fibre analysis was done within 5 days of sample collection; the 24 hourly samples were all analysed within 30 days of sampling.

3.4 RESULTS

3.4.1 ESTIMATE OF DIFFUSIVITIES

The diffusivities of chlopyrifos and endosulphan in water were estimated using Equation 32. The required parameters for chlorpyrifos and endosulphan, and the resulting diffusivities, are given in Table 3.1.

Table 3.1: Calculation of diffusivities in water, at 20 ^{o}C (298K)

Parameter	Units	Water	Chlorpyrifos	Endosulphan
Molecular Mass, M_B (water)		18.0		
Temperature	K	298		
Viscosity, μ_B (water)	cP	0.993		
Association Parameter, ϕ_B (water)		2.26		
Molal Volume, V_A	cm^3/g		310-320	320
Calculated Diffusivity (Equation 31)	**cm^2/s**		**$0.44*10^{-5}$**	**$0.44*10^{-5}$**
Calculated Diffusivity	**m^2/s**		**$0.44*10^{-9}$**	**$0.44*10^{-9}$**

In order to obtain a perspective on the validity of these calculated estimates of the diffusivities, they may be compared with the experimental values, in m^2/s units, for ethanol ($0.84*10^{-9}$), benzyl alchohol ($0.82*10^{-9}$), acetic acid ($1.21*10^{-9}$) and sucrose ($0.52*10^{-9}$).[16] The calculated values fall within the expected range of values for diffusivities in water at infinite dilution. Note that the diffusivity varies in direct proportion to the absolute temperature. For small changes in temperature, the variation in diffusivity is negligible. For a 5 ^{o}C variation in temperature, the change is less than 2%.

3.4.2 DETERMINATION OF THE DISTRIBUTION CONSTANT (K_{FS}) VALUES

The experimentally estimated Kfs values and the literature values are presented in Table 3.2.

Table 3.2: Literature and measured (at 20 °C) K_{fs} values

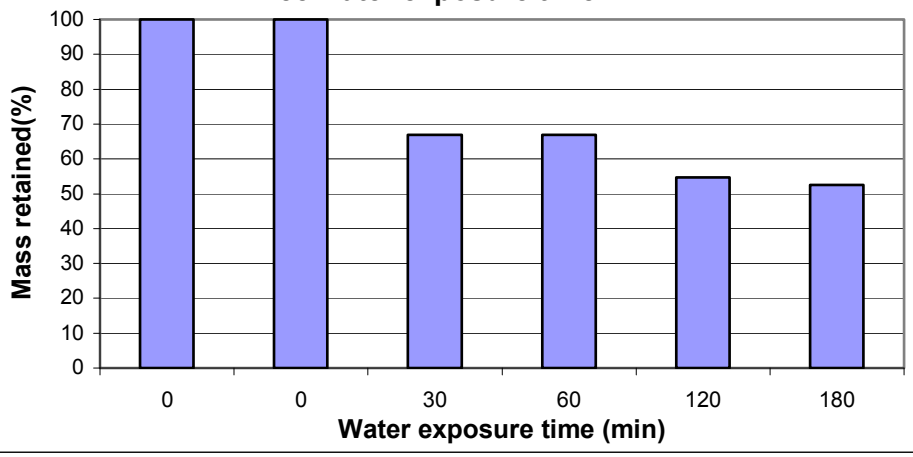
Analyte	Chlorpyrifos	Endosulfan I	Endosulfan II	Endosulfan Sulphate
K_{fs} (literature values)[17]	(no value)	25000	10000	400
K_{fs} (experimental)	**520-580**	**2300-2900**	**1200-2300**	**420-620**

The exposure time of 60 minutes used in these experiments was greater than the equilibrium extraction times of 45 minutes quoted by MacGillivray[19]. Note that MacGillivray does not quote the temperatures at which the values were measured. Thus a direct comparison is not possible but the difference between the measured Endosulfan values and the literature values appear to be greater than that due to possible temperature differences and non-equilibrium conditions. Large differences in published K_{fs} values have previously been reported.[18]

3.4.3 ASSESSMENT OF ANALYTE RETENTION ON THE FIBRE

The results, for the fibre protruding by 3mm from the end of the steel shaft, exposed to a 1ug/l spiked standard for 15 minutes and then to analyte free water, are shown in figure 3.11.

Figure 3.11: Mass chlorpyrifos (1ppb) retained vs analyte-free water exposure time



3.4.4 LINEARITY OF MASS ABSORBED WITH RESPECT TO EXPOSURE TIME AND CONCENTRATION

The linearity of mass absorbed with respect to exposure time is a function of the Biot number:

$$Bi = \frac{k_c.x_1}{K_{fS}.D_f} = \left(\frac{D_s}{D_f}\right)\left(\frac{x_1}{\delta_f}\right)\left(\frac{1}{K_{fS}}\right) \qquad \ldots\ldots. (23)$$

The literature K_{fs} values for the pesticides of interest vary considerably, from 400 to 25 000.

The calculation of the experimental Biot numbers (using Equation 23) requires an estimate of δ_f, the effective film thickness. If the fibre is positioned flush with the steel sheath, Equations 32 and 33 may be used to estimate the film thickness. For velocities in the range 0.01 to 0.5m/s (0.01 m/s corresponds to a slow moving stream) the film thickness ranges

from 0.18mm to 0.025mm. If the fibre is retracted to a distance 0.5mm from the rim of the sheath, the film thickness is effectively 0.5mm plus δ_f.

The system parameters are shown in Table 3.3. The Bi values for a range of parameter values are given in Table 3.4 and Figure 3.5.

Table 3.3: System parameters

Parameter	Units	Value
Fibre diameter	[m]	$0.300*10^{-3}$
Sheath cross-sectional area	[m^2]	$7.07*10^{-8}$
Fibre cross-sectional area	[m^2]	$6.12*10^{-8}$
Diffusivity in water: D_s (average)	[m^2/s]	$4.2*10^{-9}$
Diffusivity in polymer: D_f (=1/6Ds)	[m^2/s]	$0.7*10^{-9}$
Fibre length, x1	[m]	0.010
Water density (20 ^oC)	[kg/m3]	998
Water viscosity	[N.s/m2]	0.001

Table 3.4: Biot Number vs Kfs and calculated film thickness

Velocity u_∞[m/s] →	0.01	0.05	0.1	0.5	Fibre Retracted by 5.0E-4m, u_∞= 0.1	Initial Bi case
Film thickness, □f[m] →	1.8E-04	8.0E-05	5.6E-05	2.5E-05	5.6E-04	1.0E-02
Kfs	**Biot**	**Biot**	**Biot**	**Biot**	**Biot**	**Biot**
400	1.402	3.134	4.433	9.912	0.449	0.025
1000	0.561	1.254	1.773	3.965	0.180	0.010
6000	0.093	0.209	0.296	0.661	0.030	0.002
10000	0.056	0.125	0.177	0.396	0.018	0.001
15000	0.037	0.084	0.118	0.264	0.012	0.001
20000	0.028	0.063	0.089	0.198	0.009	0.001
25000	0.022	0.050	0.071	0.159	0.007	0.000

In general, the Biot Number may fall in the range 0.1 to 10, depending on the values of the various parameters in the equation, indicating that the lumped parameter models (Bi<0.1 or Bi>10) are not necessarily good approximations.

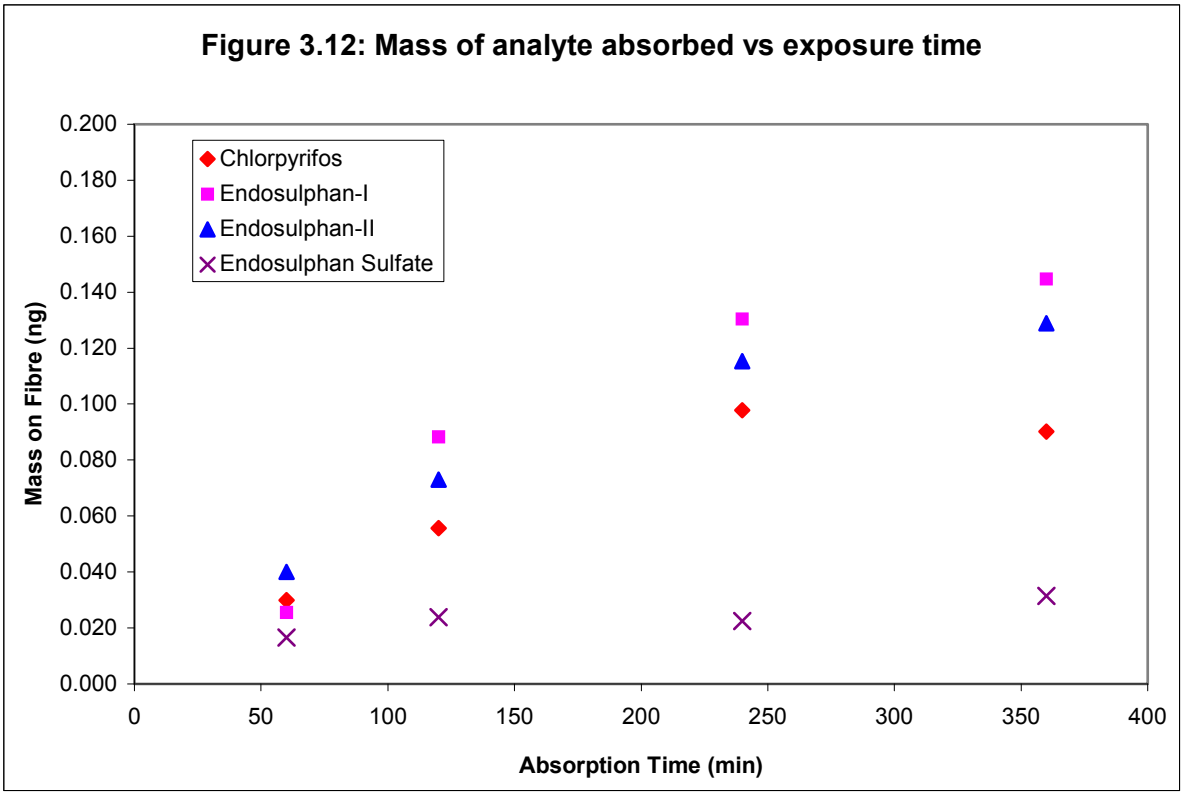
The Biot numbers for the experimentally determined K_{fs} values, and the experimental velocity of 0.1m/s are given in Table 3.5.

The mass of analyte absorbed with exposure time is shown in Figure 3.12.

Table 3.5: Experimental Kfs and Biot numbers

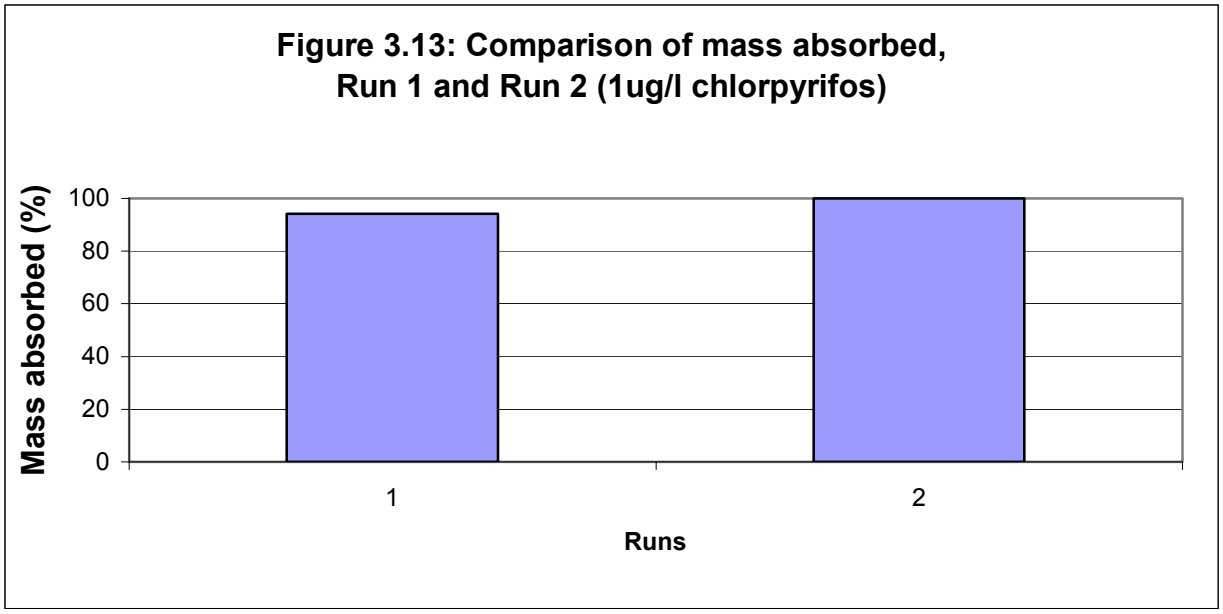
Analyte	Kfs Values		Calculated MT Biot numbers	
	Literature	Measured	Based on Literature Kfs	Based on Measured Kfs
Chlorpyrifos	-	520-580		3.1 – 3.4
Endosulfan I	25 000	2300 - 2900	0.07	0.6 – 0.8
Endosulfan II	10 000	1200 - 2300	0.18	0.8 – 1.6
Endosulfan Sulphate	400	420 - 620	4.2	4.4 – 6.9

Figure 3.12: Mass of analyte absorbed vs exposure time



3.4.5 EXPOSURE TO DIFFERENT CONCENTRATION PROFILES

The results of the mass absorbed using two concentration profiles are given in Figure 3.13. The results for the two concentration profiles are within 6% of each other.



3.4.6 COMPARISON BETWEEN 24 X 1-HOUR GRAB SAMPLES AND THE 24 HOUR TWA SAMPLE

Figure 3.14 presents the results of the 24 autosampler grab samples, taken hourly at the sampling point Bdr, over the period 28/29 March 2002.

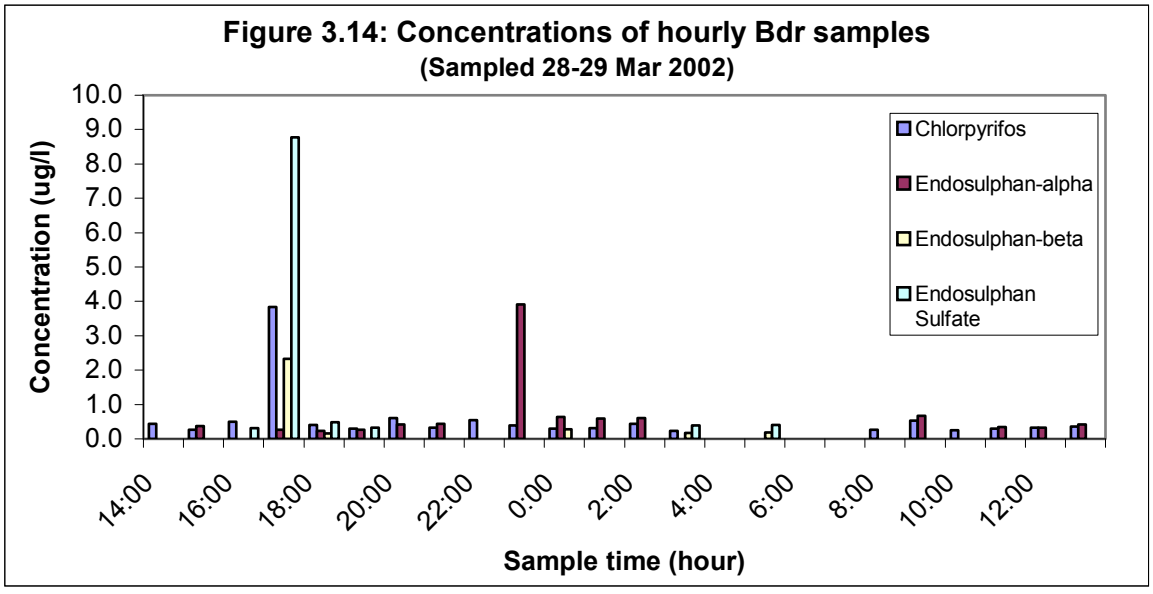


Table 3.6 contains the results of the 24-hourly field samples, and the means and standard deviations of the 24-hourly values.

Table 3.6: Hourly Pesticide Concentrations at BDr, 28 March 2002

Sample	Sampling Time	Chlorpyrifos Concentration (ug/l)	Endosulphan-alpha Concentration (ug/l)	Endosulphan-beta Concentration (ug/l)	Endosulphan Sulfate Concentration (ug/l)
BDR 1	14:00	0.435	0.000	0.000	0.000
BDR 2	15:00	0.260	0.372	0.000	0.000
BDR 3	16:00	0.501	0.000	0.000	0.314
BDR 4	17:00	3.841	0.272	2.332	8.781
BDR 5	18:00	0.406	0.230	0.159	0.488
BDR 6	19:00	0.292	0.270	0.000	0.325
BDR 7	20:00	0.608	0.417	0.000	0.000
BDR 8	21:00	0.329	0.434	0.000	0.000
BDR 9	22:00	0.538	0.000	0.000	0.000
BDR 10	23:00	0.395	3.916	0.000	0.000
BDR 11	00:00	0.295	0.643	0.277	0.000
BDR 12	01:00	0.314	0.594	0.000	0.000
BDR 13	02:00	0.438	0.603	0.000	0.000
BDR 14	03:00	0.227	0.000	0.178	0.388
BDR 15	04:00	0.000	0.000	0.000	0.000
BDR 16	05:00	0.000	0.000	0.186	0.410
BDR 17	06:00	0.000	0.000	0.000	0.000
BDR 18	07:00	0.000	0.000	0.000	0.000
BDR 19	08:00	0.268	0.000	0.000	0.000
BDR 20	09:00	0.534	0.668	0.000	0.000
BDR 21	10:00	0.247	0.000	0.000	0.000
BDR 22	11:00	0.300	0.340	0.000	0.000
BDR 23	12:00	0.324	0.319	0.000	0.000
BDR 24	13:00	0.365	0.420	0.000	0.000
	Mean:	0.45	0.40	0.13	0.45
	Standard Deviation:	0.74	0.79	0.48	1.78

Figure 3.14 clearly shows the diurnal variability and range of the concentrations.

The TWA sample was obtained over the same sampling period, with the sampling pump set to give a flow velocity of 0.1m/s past the SPME fibre positioned flush with the steel shaft. Under these conditions the Biot numbers, based on literature values for K_{fs}, were in the range 0.2 to less than 0.01 for 3 of the analytes, but greater than 4 for endosulfan sulphate. The Biot numbers based on the experimentally determined K_{fs} values range from 0.8 to 4.4. If the latter Kfs and Biot numbers values are correct, the mass absorbed into the fibre would approach equilibrium rapidly and the TWA would be underestimated.

These Biot values and the Biot values listed in Table 3.4 emphasize that a single fibre system cannot yield good precision and linearity for the simultaneous sampling of analytes with very different K_{fs} values.

The mass of analyte absorbed into the fibre was determined by comparison with a direct injection calibration curve using external standards. Table 3.7 summarises the mass absorbed of the different analytes.

Table 3.7: Mass of analyte absorbed into during 24h TWA sampling

Analyte	Chlorpyrifos	Endosulfan I	Endosulfan II	Endosulfan Sulphate
Mass absorbed (m_T)[pg]	0.28	0.19	0	0
Corrected mass (m_T)[pg]	0.52	0.36	0	0

The mass loading rate is given by:

$$\frac{dm}{dt} = \frac{A.D_s}{\Delta x}\left(C_s - C_i\right) = R.(Cs - Ci) \qquad \ldots\ldots\ldots (8)$$

The total mass loaded into the fibre is then:

$$m_T = R.\int_{t=0}^{t_T} [C_{s\infty}(t) - C_i(t)]dt \qquad \ldots\ldots (29)$$

The factor R may be calculated with A= $7.1*10^{-8}$ m^2, D_s = $0.44*10^{-9}$ m^2/s and $\Delta x = \delta_f = 5.6*10^{-5}$ m (Tables 1 and 2); hence the initial volumetric sampling rate, R = $0.56*10^{-12}$ m^3/s. In the absence of experimental mass sampling rates to define the rate of decrease in sampling rate with time over the sampling period, the initial sampling rate R was used to estimate C_{TWA}:

$$C_{TWA} = m_T/(R.t_T);\ t_T = 24 \text{ hours};\ t_T = 8.64*10^4 \text{ s}$$

Table 3.8 compares these estimates against the 24 hours mean values of Table 3.6.

Table 3.8: Comparison TWA sample concentrations vs average of 24 h grab samples

Analyte	Chlorpyrifos	Endosulfan I	Endosulfan II	Endosulfan Sulphate
C_{TWA}[ug/l]	0.01	0.01	0	0
Ave. of 24xhourly samples [ug/l]	0.45	0.45	0.40	0.13

The TWA sample clearly under-predicts the average values as measured using the 24 hourly grab samples over the sampling period. In the case of Endosulfan II and Endosulfan Sulphate, the TWA sample method failed to detect the analytes. The most probable reason for the under-prediction of the actual concentrations is the loss of analyte from the fibre during periods of low concentration. This problem (if confirmed) may be addressed by de-rivitisation. The second contributing factor to the under-prediction of the TWA concentration is that the configuration used – the flush mounted fibre – does not exhibit linearity (with respect to mass absorbed-time) beyond about 4 hours for 3 of the 4 analytes, as shown in Figure 3.12.

3.5 DISCUSSION

The theoretical analysis indicates that the rate of absorption of analytes is approximately constant, for a constant sample concentration, over a time period that may be predicted based on the Biot Number of the system. Conversely, the conditions required for linearity may be predicted using the theoretical analysis. The limited TWA data generated during this project (Figure 13) appear to confirm this result over a two-hour period. However a much

more extensive experimental program is required to confirm (or contradict) the possibility of developing a practical and cost effective system for obtaining a TWA sample in water.

To date, the reported uses of SPME fibres to obtain the TWA concentration of environmental samples have been confined to the measurement of various analytes in air, based on a simplified theoretical model that is valid for fairly restrictive conditions. In particular, the simplified model assumes that the fibre face represents a 'zero sink', and that the flux of analyte to the fibre, and therefore the mass transfer or mass loading rate, is proportional to the free stream sample concentration and the time of exposure. The 'zero sink' assumption confines the method to analyte loading into the fibre of <5% of the equilibrium value. This approach would not give useful results for the analysis of sample concentrations less than 20 x the method detection limit (the factor 20=100/5) using standard SPME techniques since the mass absorbed would be less than that required for quantitationThe detection limits (in water) for the pesticides of interest for this study are of the order of 0.02 to 0.05 ug/l. Thus an approach based on the assumption that the concentration at the interface is approximately <5% would have a detection limit of at least 20x these values, or 0.4 to 1.0ug/l. In practice, the detection limits would be even higher due to additional sources of variability inherent to the TWA methods. Since the concentrations of interest are of the order of 1ug/l, a method based on the 'zero sink' assumption would have limited value.

On the other hand, allowing the interfacial concentration to increase to 20 or 30% of the equilibrium value, the concentration driving force is correspondingly reduced. If linearity is maintained during the sampling period, the effect of the reduced driving force (and hence sampling rate) may be estimated but this complicates the experimental procedures and introduces additional sources of experimental error.

The theoretical analysis shows that, in the case of reversible absorption/ desorption, if $Cs\infty$ is less than C_{si}, desorption will occur and the mass accumulated in the fibre will not reflect the TWA concentration. This observation holds irrespective of whether or not the 'zero sink' assumption (Csi~0) is invoked. As far as can be ascertained through the literature search, this limitation of the use of SPME fibres for TWA sampling has not been explicitly recognised to date. Derivitisation would ensure that the reverse process (desorption) does not take place. A rigorous experimental program would be required to demonstrate that the mass loading rate remains constant (or at least decreases linearly with respect to time in accordance with Equation 8) under a defined set of experimental conditions.

3.6 CONCLUSIONS

The theoretical analysis presented in this report showed that the SPME system could be adapted to obtain a TWA sample of pesticides in water, at concentrations of the order of 1 ug/l. The system could be operated with mass loadings of 20-30% of the equilibrium values, with a corresponding increase in method sensitivity (detection limit) providing that the linearity of the mass loading rate through the sampling period was preserved by operating the system at sufficiently low Biot number for the sample period under consideration. The theoretical analysis also demonstrated the impossibility of satisfying both linearity and sensitivity criteria with a single fibre located in a particular configuration if the analytes of interest have a wide range of K_{fs} values – in this case the ratio of the highest to lowest K_{fs} values, based on literature data, is approximately 50:1. A multi-fibre system is required for these cases.

The temperature dependence of K_{fs} values may be significant, therefore in-field temperature control of the fibre may be necessary for maximum reproducibility.

The preliminary experimental results confirmed that in principle and in a practical environmental setting, the SPME device could be adapted to obtain a TWA sample of the pesticides of interest, over a sampling period of several hours. The field test showed that a TWA sample could be obtained with minimal effort over a comparatively short sampling period of 3 hours. However, the TWA sample result significantly underestimated the average concentrations over a 24-hour sampling period, compared with the average of 24 hourly samples taken over the same period.

The laboratory based experiment showed that a similar (agreement within 6%) TWA sample was obtained for two markedly different exposure patterns with the same theoretical TWA concentration, a key requirement for the success of the method. However, this test does not confirm that the absolute value of the measured concentration is accurate, and it is thus not a definitive test of the validity of the result.

The fibre fails to retain, for the time period required, 100% of the analyte absorbed, with losses over 50% over a 3-hour period. The losses over a 24-hour period may be as high as 90%. Based on limited experimental data, this loss factor was assumed to be constant, and the calculated TWA result was adjusted accordingly. The validity of this calculation procedure has yet to be confirmed experimentally. The loss of analyte clearly degrades the sensitivity (and possibly the reproducibility as well) of the method. The loss of analyte from the fibre may be overcome by in-fibre derivitisation, or chemically fixing the absorbed analyte, but this work was beyond the scope of the present project.

The linearity of the mass loading rate, under restrictive experimental conditions, over a sampling time of two hours was confirmed, within experimental error.
Insufficient experimental results have been obtained to date to provide estimates of the accuracy and sensitivity of the TWA sampling method. Further work is required to determine the TWA sample method detection limit and to delineate the limitations of the method. On balance, the drawbacks of the method – the loss of analyte during periods of low sample concentration, the variation of K_{fs} with temperature and the different response characteristics of fibre-coating/ analyte systems (K_{fs} values) – may nullify its advantages. The possibility of using the SPME fibre system to obtain peak concentrations over a given period may be more promising.

The prototype field sampling system that was used is essentially simple. It consists of a custom-made fibre holder and a small sampling pump. A robust standardised (or commercial) system costing less than R10 000 could be developed. This cost should be compared against the cost of an electronically and mechanically more complex autosampler, namely about R50000 (2002 price).

The primary purpose of obtaining the 24x hourly samples, using the autoasampler, was to provide the basis of comparison for the SPME Fibre TWA sample. However, analysis of these 24 samples yielded an important observation. The 24x hourly grab samples revealed that the diurnal variation in analyte concentration is considerable, confirming that a single grab sample of a contaminated stream or river is very likely to over or underestimate the average value over a given day. A single grab sample would therefore provide a poor estimate of the time-averaged concentration of the stream sampled. The uncertainty is even greater if grab samples are taken less frequently than daily, as is usually the case. Thus, regardless of the precision of the analytical method applied to the (single) grab sample, an assessment of the level of contamination of the stream is subject to the far greater uncertainty because the grab sample provides a poor representation of the time averaged concentration than uncertainty due to lack of analytical precision. This uncertainty has obvious implications for health, environmental risk assessments and water resource management decisions that have of necessity to be based on the available data.

Over the past few decades, considerable resources have been devoted to improving analytical methods for the detection and analysis of pesticides in environmental media. This work has highlighted the need to shift the focus to the problem of obtaining a time-representative sample. A sample that provides a more accurate representation of contamination levels clearly provides a better basis for the assessment of the potential public health and environmental impact of these contaminant levels, and that it therefore provides a better scientific basis for environmental decision-making and management. This warrants the allocation of significant resources to enable the rapid development of a sampling system capable of yielding an accurate TWA result.

The prototype system described in this report, or any viable alternative sampling system capable yielding a TWA result of similar accuracy and precision to existing grab sampling (followed by laboratory analysis) methods, applicable to a wider range of pesticides, should be developed. In principle, a system could be developed that is capable of yielding the maximum concentration during a given time period as well as the TWA concentration.

The problem of time-representative sampling appears to be universal and such a research program may therefore be expected to evoke international interest. The results of this work may be used to delineate the objectives and resources required of such a research program.

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Chapter 4

ENZYME-LINKED IMMUNOASSAYS

CONTENTS

4.1 LITERATURE REVIEW

Enzyme-linked Immunoassays are tests based on the reaction of selective antibodies attached to solid supports with sensitive enzymes and analytes. They are capable of detecting low levels of pollutants with high specificity, for example chlorpyrifos detection ranges from 0.1µg/l to 3.0µg/l and endosulfan (as mixed isomers) from 0.08µg/L to 1µg/L. Field kits can be used in evaluating contamination sites as well as monitoring worker safety. Regulatory requirements for handling issues such as worker safety could easily be monitored with field test kits[1]. ELISA is being used in a number of applications. It is currently being used in the analysis of animal drug residues in food[2] and immunoassays continue to play a major role as diagnostic tools in clinical chemistry[3]. Immunoassay methods vary depending on the compound or compounds they are capable of detecting.

Enzyme-linked competitive immunoassay (ELISA) is one of the most common formats of pesticide immunoassays. There are two variations that are commonly in use, the one uses an immobilizing antibody and the other an immobilizing conjugate on a solid-phase support such as a 96-well microtiter plate. The coating conjugate on the microtiter plate must differ from the immunogen to prohibit binding from antibodies that recognize the carrier protein[4].

The one format using immobilized conjugate is based upon the competition between the immobilized conjugate and the analyte in the coated well. The analyte blocks the binding of the antibody to the plate. In a precoated well, equal volumes of antibody and analyte are added. The mixture is incubated for a period of time, and the liquid phase is washed away. The antibody that is bound to the plate is measured. If the antibody was conjugated to an enzyme before reacting with the analyte, then the final step is to add substrate and measure absorbance of the resulting colour. If the enzyme was not conjugated to the antibody first, then the second antibody with an enzyme tag is added[5].

ELISA holds the promise of decreased sample processing, high compound specificity and significant increase in the number of samples that can be analyzed compared to traditional analytical techniques.[1] The assays can be class-specific (they are able to screen for a certain class of compounds), or compound-specific (they have a high specificity for the compounds of interest). Other substances in water and soil can affect ELISA determinations. False positive results may be caused by interference from structurally similar compounds or natural substances that weakly interact with the antibody but are present at a high concentration to give a false positive value with the assay. In situations such as these, the concentration of the target compounds is uncertain and in any case confirmation by alternative techniques is needed. Using traditional methods such as SPE (Solid phase extraction) or LLE (Liquid-liquid extraction) before ELISA may reduce the problems of false positives[6].

The application of ELISA for the determination of organic compounds in highly contaminated soils has grown in the last few years due to the need to develop rapid methods for the determination of contamination of soils following possible spills. Oubina et al[7] reported on the evaluation of Rapid ELISA in different waters. They set out to apply ELISA test as a first screening device for the determination of chlorpyrifos-ethyl in a highly contaminated soil. Final determination and confirmation of the results obtained were achieved using LC/DAD (Liquid chromatography/Diode array detection) [5].

Oubina et. al.[7] observed no significant differences between filtered and non-filtered estuarine waters with or without prior acidification. An estuarine system is a semi-closed body of water having a free connection with open sea and within which seawater is measurably diluted with fresh water runoff. When the direct ELISA measurement was applied, results indicated absence of false negatives in the estuarine waters. In another experiment an acid pH was

used. No difference between acidified and non-acidified samples was observed. They concluded that the ELISA test could be applied in various environmental situations, as it was not affected by acidification of samples at the concentration levels studied[6].

There has been an increase in the use of immunoassays as an analytical tool for the detection of pesticides[1]. Immunochemical assays for pesticides are not a replacement for traditional analytical techniques such as gas chromatography (GC), but they are very useful in quantifying pesticide residues when chromatography methods, which are difficult and time-consuming, cannot be performed. Immunoassays are currently viewed as an important tool for analysing large volumes of samples[1].

The ELISA method has been used internationally for the determination of a wide range of analytes in various matrices[7-9].

For example, analysis of Canadian strawberries for benomyl residues using ELISA and High Pressure Liquid Chromatography (HPLC) found that the precision for the ELISA kit was not as good as HPLC and that the agreement of quantitation for the two tests was significantly different[7]. It was recommended that positive ELISA results should be confirmed with HPLC.

In a New Zealand national survey, two ELISA atrazine test kits were compared to gas chromatography mass spectrometry (GC-MS) for testing 28 groundwater samples[8]. The one test kit, frequently used in monitoring, had a relatively high detection limit and only showed four positive detections. The other, high sensitivity (HS) kit, detected pesticides in twenty of the samples of which nineteen were confirmed with GC-MS. It was concluded that the HS kit was a good indicator of pesticide contamination in the water samples.

The general opinion about ELISA is that although it is a fast semi-quantitative tool for screening a large number of samples, the results must be validated and verified by using established methods of analysis such as GC-MS or HPLC .

4.2 MATERIALS AND METHODS

Field sample sites are described in chapter one, section 1.4
Endosulfan and chlorpyrifos ELISA's are in the form of plate kits

4.2.1 PRINCIPLE OF OPERATION

The plate kit uses antibodies that bind an analyte (endosulfan or chlorpyrifos) and an analyte-enzyme conjugate. The analyte in the sample competes with the analyte-enzyme conjugate for a limited number of antibody binding sites. Antibodies that bind the analyte are held (immobilized) to the inside of the test wells.

Each well has the same number of antibody sites and therefore receives the same number of analyte-enzyme conjugate. Therefore, a sample with a low concentration of an analyte allows the antibody to bind many analyte-enzyme conjugate molecules. A high concentration of an analyte allows fewer analyte-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution. (Colour is inversely proportional to analyte concentration)

Appendix 4.1 gives an illustration of the principal of operation of the ELISA assay using a rapid assay as an example.

4.2.2 CHARACTERISTICS

The plate kit does not completely differentiate between analyte and structurally related compounds but detects their presence to differing degrees. Table 4.1, which lists the different ELISA plate kits for endosulfan and chlorpyrifos available from suppliers, indicates that the detection limits of the kits are fairly low[10].

Table 4.1 : Supplier's detection limits

Compound	DL (detection limit - ppb) Supplier's data
Endosulfan (mixed isomers)	0.08
Endosulfan-alpha	0.07
Endosulfan-beta	0.08
Endosulfan Sulfate	0.08
Chlorpyrifos-Ethyl	0.05
Chlorpyrifos-Methyl	0.02

4.2.3 TESTING PROCEDURE

The ELISA testing procedure was according to that described in the manual:[10]

1. The standard procedure adopted, for both endosulfan and chlorpyrifos kits, was to run 2 strips with deionized water as the negative control, 3 calibrators and samples in duplicate.
2. For the endosulfan kit: 150µl of negative control (deionized water), 150µl of each calibrator (0.075, 0.25, 1ppb) and 150µl of each sample was added to each well. For the chlorpyrifos kit: 100µl of negative control (deionized water), 100µl of each calibrator (0.05, 0.3, 1ppb) and 100µl of each sample was added to each well.
3. 50µl of the working endosulfan-enzyme conjugate was added to each well in the endosulfan plate kit. 100µl of chlorpyrifos enzyme conjugate was added to each well in the chlorpyrifos plate kit.
4. The contents of the wells were thoroughly mixed, by moving the strip holder in a rapid circular motion on the bench top for approximately 1 minute.
5. The wells were covered with tape to prevent evaporation and it was incubated at ambient temperature for 1hour. During incubation, the use of an orbital mixer was employed, operating at a set speed of 200rpm.
6. After incubation, the cover was gently removed and the contents were discarded from the wells into a sink. The wells were completely flooded with cool running tap water, and then emptied by shaking. This wash step was repeated 5 times. The plate was inverted and tapped to remove as much water as possible.
7. For both the endosulfan kit and chlorpyrifos kit: 100µl of substrate was then added to each well, starting with the negative control, then the calibrators and ending with the samples.
8. Wells were covered with new tape and incubated at ambient temperature for 30 minutes. Once again the use of an orbital mixing device was employed, operating at 200rpm.
9. For both the endosulfan kit and chlorpyrifos kit, 100µl of stop solution was then added to each well and thoroughly mixed. This turned the solution yellow in the wells. The plate was read within 30 minutes of adding the stop solution. The stop solution, which was 1N HCl, was handled with care.

10. The plate was placed in the automated plate reader. Results were obtained within approximately 3 seconds. The wavelength of the microtitre plate reader was 450 nanometers. (Absorbance was measured).
11. For both endosulfan and chlorpyrifos, the mean of 2 optical densities (OD) of each component in the wells was calculated and the reciprocal plotted against concentration to produce the ELISA calibration curve. (a plot of 1/OD versus concentrion in ppb)

4.2.4 TESTS PERFORMED ON ELIZA KITS

Four tests were performed to evaluate the ELISA plate kits.

TEST 1

Field samples were taken on 6/12/01 and the ELISA kits used to determine chlorpyrifos and total endosulfan concentrations .

TEST 2

Field samples were again taken on 13/02/01 and the ELISA kits used to determine chlorpyrifos and total endosulfan concentrations .

TEST 3

The field samples of the 13/02/01 were analysed by both the ELISA method and SPE / GC method to compare the results .

TEST 4

Determination of ELISA standards concentration using prepared standards for calibration by SPME

Solid phase microextraction / Gas Chromatography (SPME/GC) runs were performed with prepared mixed standard and the ELISA standard in order to observe how well the two standards compared. The only ELISA standard used was for endosulfan, because the chlorpyrifos ELISA standard had expired .

Experimental Description of TEST 4

A run was performed using the prepared mixed standard as the known standard to draw a calibration curve, and the ELISA standard as the unknown. The purpose was to determine the concentration of the ELISA standard as an unknown and then compare the experimentally determined concentration value with the theoretical concentration value. The prepared mixed standard contained three isomers of the analyte endosulfan (alpha, beta and sulfate), made up in equal quantities. The ELISA standard, contained three isomers of endosulfan (alpha, beta and sulfate).

Room temperature was maintained at 20^{o}C, with the GC Nitrogen (Carrier Gas) pressure set at 430kPa for the duration of the experimental analysis. For each individual analysis, the prepared calibrators, as well as the unknown ELISA standards, were contained in a 30ml baffled glass vial, with a Teflon coatedstirrer bar, containing a cap with a Teflon coated septum, at a volume of 25ml. For each individual analysis, the solution in the glass vial was stirred for an initial period of 4 minutes in order to allow for even bulk mixing. The SPME fibre was then exposed to the 25ml solution in the glass vial for an absorption time of 25 minutes, after which the fibre was removed and placed into the GC injection port to be

desorbed for a period of 5 minutes, after which it was removed. The duration of each individual GC cycle analysis was 25 minutes. The areas of each component in the prepared calibrators and unknown ELISA standards were recorded, and these areas were used to draw the endosulfan standard calibration curve.

4.3 RESULTS

4.3.1 FIELD RESULTS

Table 4.2 ELISA field results

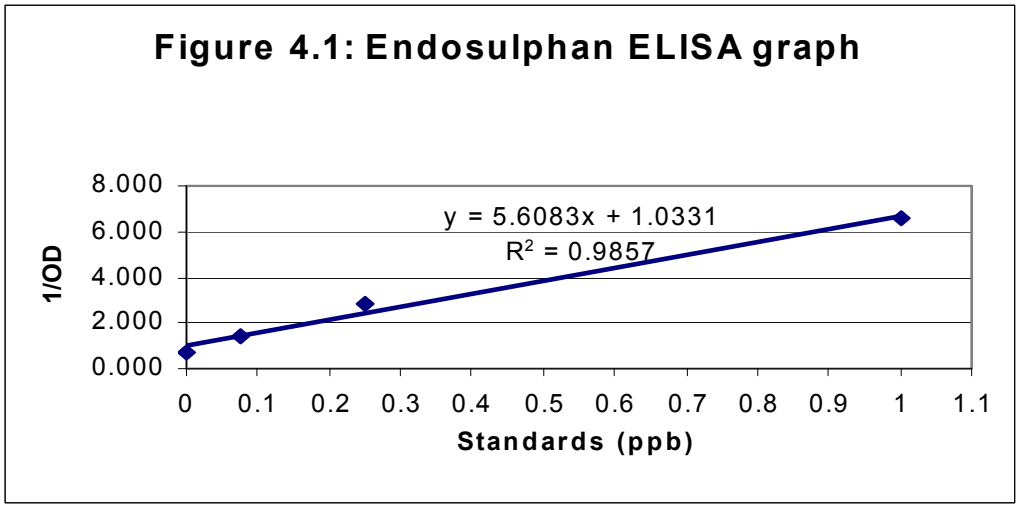
	TEST 1 (6/12/01)		**TEST 2** (13/02/01)	
	Endosulfan	Chlorpyrifos	Endosulfan	Chlorpyrifos
SAMPLE	Conc.(ppb)	Conc.(ppb)	Conc. (ppb)	Conc. (ppb)
Dd	0.13	0.03	0.27	<DL
Br1*			ND	0.41
Br2*	ND	0.24	ND	0.64
F	0.11	0.05	0.28	ND
Bdr	0.22	0.24	0.39	0.50
G1*	ND	0.02	ND	ND
G2*			0.27	ND
Ar1*	ND	0.02	ND	ND
Ar2*	ND	0.03	0.09	0.18
Cdr	ND	0.02	ND	ND

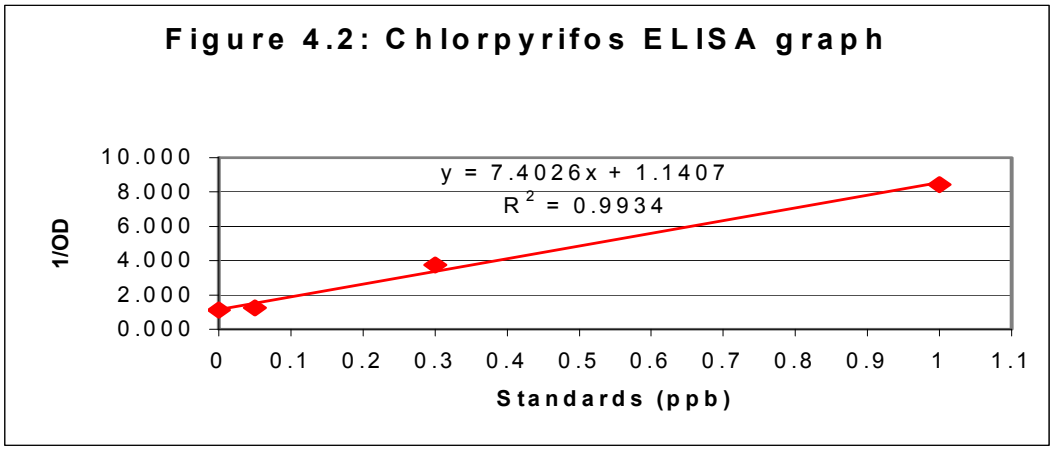
* Certain sites were sampled twice
Note: ND = NOT DETECTED .

Table 4.2 shows consistent chlorpyrifos and endosulfan contamination in the ppb range detected by ELISA in most sites sampled. Pesticide levels on the 13/02/2001 were generally higher than those measured on the 6/12/01.

4.3.2 CALIBRATION GRAPHS

The following figures show an example of a chlorpyrifos and endosulfan calibration curve.





TEST 3

Table 4.3: Comparison of ELISA and solid phase extraction (SPE) field results

	Chlorpyrifos	Chlorpyrifos	Endosulfan[#]	Endosulfan
	SPE Concentration (ppb)	EL:ISA Concentration (ppb)	SPE Concentration (ppb)	EL:ISA Concentration (ppb)
Br1*	0.1	0.41	0.02	ND
Br2*	0.42	0.64	ND	ND
Bdr	0.02	0.5	ND	0.39
Cdr	ND	ND	ND	ND
F	1.16	ND	0.22	0.28
G	ND	ND	ND	ND
Ar1*	ND	ND	ND	ND
Ar2*	0.01	0.18	ND	0.09
Dd	ND	ND	ND	0.27

* Certain sites were sampled twice
[#] Total endosulfan

Table 4.4: Qualitative comparison of ELISA and SPE detections of chlorpyrifos and endosulfan

	Chlorpyrifos	Chlorpyrifos	Endosulfan[#]	Endosulfan
	SPE Concentration (ppb)	EL:ISA Concentration (ppb)	SPE Concentration (ppb)	EL:ISA Concentration (ppb)
Br2	+	+	+	-
Br1	+	+	-	-
Bdr	+	+	-	+
Cdr	-	-	-	-
F	+	-	+	+
G	-	-	-	-
Ar1	-	-	-	-
Ar2	+	+	-	+
Dd	-	-	-	+

* Certain sites were sampled twice
[#] Total endosulfan

Table 4.3 and 4.3 show the comparison of the results of the field samples collected on the 13/01/2001 using ELISA and solid phase extraction (SPE) methods. Because ELISA, unlike SPE, do not distinguish between different endosulfan isomers, the sum of the SPE results for the 3 isomers was used to compare to the ELISA results.

Table 4.3 shows that for chlorpyrifos the ELISA kit gave consistently higher concentrations than SPE for all the field sites except F. The percentage agreement in terms of positive or negative detections was 89%.

For endosulfan a similar trend was observed but the agreement in terms of positive or negative detections was lower, 56% .

For chlorpyrifos the ELISA method yielded no false[a] positives and for endosulfan it yielded 3 false positives. There was also one false[a] negative each for endosulfan and chlorpyrifos.

TEST 4

Table 4.6 Results of ELISA standards concentration versus prepared standards for calibration by total endosulfan concentration (3 Isomers added together)

	Concentration nominal (ppb)	Concentration Experimental (ppb)
ELISA1	1.0	0.85
ELISA2	1.0	0.99
ELISA3	1.0	1.01
ELISA4	0.1	0.55
ELISA5	0.1	0.59
ELISA6	0.1	0.58

Table 4.6 shows the sum of the 3 endosulfan isomers.
ELISA 1 .2 and 3 refer to triplicate analysis of a 1,0 ppb standard .
ELISA 4 , 5 and 6 refer to triplicate analysis of a 0,1 ppb standard .

For both concentrations the precision between replicate samples were good. At the higher concentration the experimental value corresponded well with the expected value but not at lower concentrations, indicating low accuracy as the detection limit is approached

4.4 DISCUSSION

The number of field results in the study were limited and there should be caution in drawing conclusions. Table 4.2, however, shows that the ELISA kits were able to frequently detect low levels (ppb) of chlorpyrifos and endosulfan contamination in field samples. The contamination detected was at a similar scale as found previously in WRC Project No. 795/1/00[11] using solid phase extraction and gas chromatography analysis, and pollution was the highest in the enclosed drain Bdr. The higher levels measured on the 13/02/2002 could be due to experimental variation, but are also consistent with the results of the previous Project[11] which found higher contamination during the period January to March.

When comparing the ELISA method to the solid phase extraction (SPE) method, qualitative agreement (present/absent) was high (8/9) for chlorpyrifos, but much lower (5/9) for endosulfan. The low percentage of false positives (17%) found in our study makes ELISA suitable for screening purposes. This would aid in reducing time and expense in analyzing samples that do not contain any pesticide. The low percentage of false negatives (11%) also ensures that only a small number of contaminated sites are undetected when using ELISAQuantitative agreement between the two methods was weak.

[a] False positives are positive ELISA detections when corresponding SPE results are negative and false negatives are no ELISA detections when SPE results are positive.

Triplicate analyses suggested good reproducibility for endosulfan estimation at both concentrations tested (range of difference = 15% at 1.0 ppb, and 7% at 0.1ppb).

During the period of analytical work using the ELISA, it was found that the reagents were safe to use but that they were not as long-lasting as mentioned in certain published literature. The ELISA method is not as time- consuming as traditional method of analysis.

In terms of capital cost, the ELISA method requires a relatively inexpensive ELISA plate reader compared to the much more expensive Gas Chromatograph (GC) used in traditional methods of analysis (i.e. SPE). A full cost appraisal of ELISA, SPE and solid phase microextraction is, however, done in Chapter 5.

4.5 CONCLUSIONS

From the tests performed, it appears that ELISA is a fairly reliable method of analyzing pesticide water pollution and a useful semi-quantitative tool. The reagents were safe to use but not long-lasting, effectively limiting the value of the method unless there are high volumes of tests required. The ELISA method could therefore be a useful screening tool for multiple samples as the method is not as time-consuming as traditional methods of analysis.

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Chapter 5:

COST APPRAISAL OF METHODS FOR MONITORING PESTICIDE POLLUTION OF WATER SYSTEMS

CONTENTS

5.1 INTRODUCTION

The primary objective of the economic evaluation of health programmes is to incorporate a consideration of resource consumption into decisions about their use[1,2]. Economic evaluation techniques such as cost analysis and cost-effectiveness analysis are designed to help decision-makers ensure that scarce public resources are used to obtain the greatest possible social benefit. Cost analysis is a method of comparing the costs of alternative methods or procedures that are assumed to have an equivalent effect. The goal of the cost analysis is therefore to find the least expensive way of achieving the outcome. When costs are discussed in economics, they usually refer to opportunity costs. The value of resources used is assessed according to the benefit that an alternative use of these resources would have[1,2]. For example, the resources that are used to treat an asthma patient could alternatively be used to prevent smoking.

Cost analyses have always been identified with issues of efficiency, cost-recovery and sustainability of programmes. There are two main types of costs, direct and indirect. Direct costs are resources consumed in the provision of an intervention. Indirect costs refer to the lost productivity suffered by the national economy as a result of factors such as illness, lack of efficiency and death[1,2]. In this study only direct costs (capital and recurrent) of different methods of monitoring pesticide pollution in water systems are estimated.

The costs and time associated with using ELISA, SPME and traditional Solid Phase Extraction (SPE) procedures are major factors in evaluating the utility of these analytical methods for monitoring pesticide water pollution. Additionally, it is important to evaluate the costs associated with pesticide measurements using integrated sampling methods which produce data of higher quality compared to grab sampling (samples collected at one time point).

Currently, pesticide water analysis in South Africa is performed using SPE and Table 5.1 lists the quoted price for analyzing water samples at various local laboratories and shows that, apart form the State Forensic Laboratory, the cost of analysis is high. Of the laboratories listed, only the Agricultural research laboratory has adequately sensitive detection limits for measuring pesticide water pollution.

Table 5.1 The cost of pesticide analysis in water samples at South African laboratories, October 2002

Laboratory (location)	Cost per sample (R)[#]		Number of pesticides[$]	Lowest DL * (µg/L)
	1 sample	> 200 samples		
State Forensic (Woodstock)	270	270	50	0.1
South African Bureau of Standards (Pretoria)	1800	900	100	1
Agricultural Research Council (Pretoria)	2000	1200	2[Φ]	0.005
CSIR (Pretoria)	1078	600	50	0.1

[#] Excludes VAT
[$] Approximate number of pesticides analysed per sample
* Detection limit
[Φ] The Agricultural Research Council laboratory quoted a price for analysing one active ingredient and a supplementary fee for every additional active ingredient per sample. Two pesticides were chosen in order to compare directly to the costs per sample obtained in the cost appraisal of ELISA, SPE and SPME at the PENTECH laboratory assuming two pesticides (chlorpyrifos and endosulfan)are monitored (refer to section 5.2)

The aims of this cost appraisal were therefore:

a) To compare the three analytical methods, 1) ELISA, 2) SPE and 3) SPME in terms of the average cost of analyses per sample. [COMPARISON ONE]
b) To compare the two integrated methods of sampling 1) 24-hour TWA SPME (called IM-TWA in subsequent discussions) and 2) 24-hour auto sampling SPME (called IM-AS in subsequent discussions) in terms of the average cost of analyses per sample. [COMPARISON TWO]
c) To consider the above costs in relation to costs for analysis of pesticides in water samples at other South African laboratories.

5.2 SCENARIO AND ASSUMPTIONS FOR COSTING

In any costing exercise of this nature, it is necessary to apply certain assumptions to a given scenario so as to facilitate interpretation of the costing information obtained. These assumptions are made explicit to improve applicability to other situations.

Table 5.2 summarizes the hypothetical scenario assumed to perform the costing.

For COMPARISON ONE: It is assumed that the sampling area is the Hex river valley, with an environmental health officer (EHO) based in Worcester, responsible for sampling rural water sources. Grab samples (single samples at one time point) are collected once weekly from the 8 sites in the Hex River Valley which had been sampled in the previous project

For COMPARISON TWO: It is also assumed that the sampling area is the Hex River Valley, with an EHO based in Worcester, responsible for sampling rural water sources. However, integrated samples using either IM-TWA or IM-AS are collected from the 8 sites in the Hex River Valley mentioned above. For IM-TWA, the EHO will be trained to set-up the sampling apparatus in the field and collect samples, while for IM-AS, it is more practical for the EHO to request the laboratory performing the analysis, to also do the sampling. There will be only one set of equipment and therefore only one site will be sampled at a time. Samples will be

collected once monthly. For IM-TWA the apparatus will therefore be set-up in the field by the EHO, collected the next day and the fibre sent by courier to PENTECH laboratory for analysis of the two pesticides, endosulfan and chlorpyrifos using SPME. For IM-AS, PENTECH will setup the apparatus in the field and collect the next day.

Table 5.2 Assumptions made for costing to compare a) 3 analytical methods and b) 2 integrated methods of sampling

Criteria	Assumptions	
	COMPARISON 1	COMPARISON 2
Sampling area	Hex River Valley	Hex River Valley
Type of sample	Grab	IM-TWA[$] & IM-AS[$]
Sampler	EHO based in Worcester	IM-TWA[$]: EHO based in Worcester IM-AS:PENTECH
Pesticides	Endosulfan and chlorpyrifos	Endosulfan & chlorpyrifos
Sampling sites	8 sites in the Hex River Valley sampled regularly in the previous project (see Table 1.1 in Chapter 1)	8 sites in the Hex River Valley sampled regularly in the previous project (see Table 1.1 in Chapter 1)
Sampling frequency	1 per week	1 per month[#]
Transport, laboratory	Courier, PENTECH	IM-TWA[$]: Courier, PENTECH IM-AS[$]: PENTECH

* For TWA, the EHO will setup and collect samples, while for sampling with the autosampler, the EHO will request the laboratory to collect samples.
[#] There will only be one set of equipment and therefore only one site will be sampled at a time; SPME will be used to analyze the samples.
[$] IM –TWA and IM-AS sampling is explained in the text (objectives)

5.3 METHODS

Cost items were grouped into capital and recurrent costs. Capital costs such a building and equipment were annualized by estimating the lifespan of the item and using an annuity factor. In order to determine costs at maximum utility levels, the time spent on capital items to perform analyses was also estimated. Recurrent costs included vehicle (transport and running costs), personnel, supplies and building operating costs. The unit cost per sample was determined by taking into account the number of samples to be analyzed.

5.3.1 COMPARISON 1: COMPARISON OF 3 ANALYTICAL METHODS

5.3.1.1 COMPARISON 1: Capital costs

(i) Building costs: Laboratory, office and storage space required for analysis of water samples using the 3 analytical methods was estimated and costs were based on building costs.

Table 5.3 Estimates of building space and time required for analyzing water samples using the 3 analytical methods, ELISA, SPME and SPE

Description	ELISA	SPME	SPE
Laboratory space (m^2)	10	20	20
Office space (m^2)	10	10	10
Estimated laboratory time required for analysis*	3 hours per month	4 days per month	8 days per month
* Laboratory time = time to perform analysis			

Total estimated building space for performing ELISA analysis with only a plate reader was 20 m^2, while for SPME and SPE, which use the relatively larger GC, the estimated space requirement was 30 m^2 (Table 5.3).
Building rates available for 1999 from Farrow Laing Ntene[3], were used to estimate current office and laboratory building costs plus 15% p.a. The 1999 rates were R 2736 per m^2 (including electrical installation, VAT) for office space and R3230 per m^2 (including electrical, air conditioning, fire protection and fittings installation, VAT) for laboratory space.

In order to estimate laboratory usage time, the time spent by the laboratory technician on analysis, explained in detail in Section 5.3.1.2 (refer to estimated personnel time), was used.

(ii) Equipment costs: Table 5.4 lists the retail price and estimated lifespan of laboratory equipment required for analysis. Thirty minutes for 8 samples would be required for GC analysis for SPME and SPE procedures. Time usage of the ELISA reader was not estimated because it would not have a major impact on the cost per sample. Lifespan of glassware for all 3 analytical methods was not factored into the cost calculations.

Table 5.4 Estimated retail price and lifespan of equipment required for the 3 analytical methods

ELISA			SPME			SPE		
Equipment	**Cost (R)**	**Lifespan (years)**	**Equipment**	**Cost (R)**	**Lifespan (years)**	**Equipment**	**Cost (R)**	**Lifespan (years)**
Reader	30000	5	GC+ECD*	400000	10	GC+ECD*	400000	10
Fridge (400L)	3000	5	Fridge(400L)	3000	5	Fridge (400L)	3000	5
Glassware	200	NE[#]	Glassware	500	NE[#]	Glassware	500	NE[#]
Computer	10000	3	Computer	10000	3	Computer	10000	3
			Holder	4000	3	Vacuum unit[$]	3025	5

[#] Not estimated
*Gas chromatograph and electron capture detector
[$]1997 price, an escalation of 15% was used to estimate current price

5.3.1.2 COMPARISON 1: Recurrent costs

(i) Transport costs: Transport costs will be the same for all 3 methods. The EHO will travel 20 km from Worcester to Hex River and back, 4 times a month. Transport costs will therefore include vehicle running costs and the cost of a courier to deliver samples to PENTECH from Worcester.

Vehicle running costs were determined using AA rates. A standard 1.6 vehicle, retailing at R120 000, was assumed. The AA fixed running costs rate (includes licensing, depreciation, insurance, interest, VAT) for a vehicle with a purchase price of R120 000 and traveling 20 000 km per annum is R1,71 per km. The AA petrol and maintenance rate for a car with a 1.6 engine capacity = R0.84 per km. Total vehicle costs per km are therefore R2.55.

Courier costs = R770 (including VAT) for 10 kg (weight of 8 samples in a holding box).

(ii) Personnel costs: These were based on the time spent by the EHO and laboratory technician to collect and analyze water samples.

The cost of employment for the laboratory technician was estimated at R120 000 per annum and for the EHO at R80 000 per annum.

The EHO will spend 4 days a month on sampling.

The time spent by the laboratory technician was estimated as follows:

- ELISA – 3 hours are required to analyze 100 samples, therefore 15 minutes is spent on 8 samples and an additional 30 minutes for computer analysis of the 8 samples, therefore the total time spent for one set of samples is 45 minutes.
- SPME – 8 hours are required to analyze 8 samples and 2 blanks, therefore the laboratory technician will spent 32 hours or 4 working days per month on analysis.
- SPE – 2 days is estimated for analysis of 8 samples, therefore 8 days in a month will be spent by the laboratory technician on analysis.

(iii) Cost of supplies: These were obtained from suppliers. Table 5.5 summarizes the retail price of supplies and the estimated number of samples that can be analyzed from the supplies.

Table 5.5 Cost of supplies and estimated number of samples analyzed

ELISA			SPME			SPE		
Material	**Cost (R)**	**Number of samples**	**Material**	**Cost (R)**	**Number of samples**	**Material**	**Cost (R)**	**Number of samples**
Plates	5500	23	Fibre	1000	100	Carbon cartridges	1300	50
			Endosulfan* standards	2000	1000	Endosulfan* standards	2000	1000
			Chlorpyrifos standards	3912	1000	Chlorpyrifos standards	3912	1000
			Methanol	300	1000	Methanol	300	1000
* Includes alfa, beta and sulfate isomers								

For ELISA the cost per plate kit is R5500 (including VAT). Therefore, with each plate having 90 wells and each sample needing replicate analyses, 32 wells would be required to analyze 8 samples for endosulfan and chlorpyrifos.

Nitrogen gas would be required for SPME and SPE methods. The cost of nitrogen gas was estimated from current usage by the PENTECH laboratory, which is approximately R1500 per annum.

(iv) Building operating costs: A building contractor was consulted to estimate maintenance costs; this was calculated at 5% of building and equipment costs.

It was estimated that electricity would be R200 per month and water would also be R200 per month.

5.3.2 COMPARISON 2: COMPARISON OF 2 INTEGRATED METHODS OF SAMPLING

5.3.2.1 COMPARISON 2: Capital costs

(i) Building: Same as SPME above.

For IM-TWA SPE, 8 days will be required for 8 samples (refer to estimated personnel time – Section 5.3.2.2, personnel costs, while for IM-AS SPME, 1.5 days X 8 samples = 12 days will be required in the laboratory.

(ii) Equipment costs:

a) IM-TWA - same as for SPME.
b) IM-AS - same as for SPME, but will also need an autosampler, priced at R4 2000 (lifespan = 5yrs).

5.3.2.2 COMPARISON 2: Recurrent costs

(i) Vehicle costs: For IM–TWA, the EHO will travel to the Hex River Valley and back, 8 times per month, therefore 160 km (20 km X 8).

The fiber will be couriered to the laboratory and back 8 times per month.

For 24-hour sampling with the autosampler (IM-AS), it was assumed that the laboratory technician will hire a vehicle for two days for setting up the apparatus in the field and collection after 24 hours, therefore 16 hiring days will be required for 8 sampling sites. The cost of vehicle hiring = R200 per day, with 150 km free petrol.

The return - trip from PENTECH to Hex Rriver = 240km, therefore the mileage for 16 days = 3840 km.

(ii) Personnel costs: For IM-TWA, it is estimated that the EHO will spent half a day per site to set up and collect apparatus and send the fiber to PENTECH. Total days per month are therefore 0.5 X8 = 4 days.

The laboratory technician will spend 1 day per site on analyzing the samples, therefore 8 days per month on 8 sites.

For 24-hour, hourly sampling, 2 days per site will be required using SPME and an autosampler = 16 days per month.

(iii) Cost of supplies: Same as for SPME (section 5.3.2.2)

(iv) Building operating costs: Same as in section 5.3.1.2 with maintenance costs estimated at 5% of building and equipment costs, electricity at R200 per month and water also at R200 per month.

5. 4 RESULTS

5.4.1 COMPARISON 1

Table 5.6 summarizes the costs of the 3 analytical methods, ELISA, SPME and SPE, while appendix 5.1 gives a more detailed account of the costs.

The cost appraisal of the 3 analytical methods found SPME to have the lowest cost per sample (R253.86), followed by SPE (R329.86), while ELISA (R425.90) had the highest cost per sample, appreciably higher than SPME.

For all 3 methods, the annualised capital cost was low and formed a low percentage (5-9%) of the total annual cost of the 3 methods.

Recurrent costs therefore formed the bulk of the costs of all 3 methods, with transport costs high for all 3 methods, and personnel costs of SPE and SPME substantially higher than ELISA. However, the cost of supplies was particularly high for ELISA, resulting in high average costs.

The bulk of the transport costs were the high courier costs, which came to R96 per sample.

The personnel costs for ELISA were substantially lower than for SPME and SPE because of shorter laboratory time. The cost of sample collection by the EHO formed a significant component of the total annualised cost of all 3 analytical methods.

The high cost of supplies for ELISA were due to ELISA plates, which came to R240 per sample for analysing chlorpyrifos and endosulfan.

If capital items were totally dedicated to the analysis of water samples for pesticides and the number of annual samples are kept at 384, then the annual capital costs of the 3 sampling methods would be: ELISA = R17 512, SPE = R73 113 and SPE = R72 868. The cost per sample for ELISA would then rise to R450.66, for SPE to R429.86 and SPME to R502.01. The implication of this would then be that in order to fully optimize the usage of capital items to achieve the low cost per sample attained in the above cost appraisal (assuming capital items are operating at maximum utility), 786 samples per annum are required for ELISA and 3200 samples per annum for SPE and SPME.

Table 5.6 Summary of costs associated with the 3 analytical methods, ELISA, SPME and SPE

Cost category/method of analysis	ELISA	SPME	SPE
RECURRENT COST			
Personnel cost (salary)			
Lab technician monthly	10,000.00	10,000.00	10,000.00
% time spent on 32 samples	1.70	18.18	36.36
Cost of time per year (384 samples)	**2,045.45**	**21,818.18**	**43,636.36**
EHO monthly	6,666.66	6,666.66	6,666.66
% time spent collecting 32 samples	18.18	18.18	18.18
Cost of time per year (384 samples)	14,545.44	14,545.44	14,545.44
Total personnel cost for 384 samples	16,590.89	36,363.62	58,181.80
Supplies			
Cost of 2 fibers (180 wells)	11,000.00		
Cost per well	61.11		
Number of wells per 8 samples	32.00		
Cost of wells for 8 samples	1,955.56		
Cost of fiber for 384 samples	93,866.67		
Total cost of supplies for 384 samples	**93,866.67**	**7,725.41**	**13,869.41**
Transport cost			
Annual vehicle cost (384 samples)	2,448.00	2,448.00	2,448.00
Cost of courier service for 8 samples	770.00	770.00	770.00
Cost of courier service per sample	96.25	96.25	96.25
Cost of courier for 384 samples	36,960.00	36,960.00	36,960.00
Total cost of transport for 384 samples	**39,408.00**	**39,408.00**	**39,408.00**
Building operating costs: maintenance			
Annual building operating cost (5%)	5,222.77	5,237.43	5,295.49
Building operating cost per sample	13.60	13.64	13.79
TOTAL RECURRENT COST	**155,088.33**	**88,734.46**	**116,754.70**
CAPITAL COSTS			
Buildings			
Total annual cost of building (including annuity factor of 8%, 30years)	5,300.18	7,730.02	7,730.02
% used	1.70	18.18	36.36
Cost of building for 384 samples	**90.34**	**1,405.46**	**2,810.92**
Cost per sample	0.24	3.66	7.32
Equipment			
Cost of GC (10 years)		400,000.00	400,000.00
Annual cost of GC (including 8%annuity factor, 10 yrs)		59,612.52	59,612.52
% time used		9.09	9.09
Annual cost of other equipment	8,320.80	1,879.70	1,635.34
Cost per sample	21.67	4.90	4.26
Cost of computer per year	44.22	44.22	44.22
Cost of computer per 1 sample	0.12	0.12	0.12
Total cost of equipment for 384 samples	**8,365.02**	**7,343.24**	**7,098.87**
TOTAL CAPITAL COST	**8,455.36**	**8,748.69**	**9,909.79**
TOTAL COST	**163,543.69**	**97,483.16**	**126,664.49**
COST PER SAMPLE	**425.90**	**253.86**	**329.86**

5.4.2 COMPARISON 2

Table 5.7 summarizes the costs of the 2 integrated methods of sampling, IM-TWA and IM-AS, while appendix 5.2 gives a more detailed account of the costs.

The costs associated with integrated sampling were much higher than for grab sampling due to the high transport and personnel cost associated with the set-up, collection, transport and analysis of samples (samples from each site have to be analyzed on separate days) for integrated sampling.

IM-TWA had virtually half the cost per sample (R865) compared to IM-AS (R1707).

For both sampling methods, the annualised capital costs were low in comparison to recurrent costs. Capital costs were higher for IM-AS because of the cost of the autosampler and the length of time in the field.

Recurrent costs of IM-AS were also substantially higher because of the high transport and personnel costs.

Table 5.7 Summary of costs associated with the 2 methods of integrated sampling 24-hour-TWA-SPE and 24-hour-autosampler-SPE

Cost category/method of analysis	TWA	Autosampler
RECURRENT COST		
Personnel cost (salary)		
Lab technician monthly	10,000.00	10,000.00
% time spent on 8 samples	36.36	72.73
Cost of time per year (96 samples)	**43,636.36**	**87,272.73**
EHO monthly	6,666.66	
% time spent collecting 8 samples	18.18	
Cost of time per year (96 samples)	14,545.44	
Total personnel cost for 96 samples	**58,181.80**	**87,272.73**
Supplies		
Total cost of supplies for 96 samples	**3,056.35**	**3,056.35**
Transport cost		
Cost of car hire for 16 days		3,200.00
Number of km traveled per month (8 samples)	160.00	3,840.00
Petrol and maintenance per km (AA rate)	2.55	0.84
Cost of transport for 96 samples	**4,896.00**	**52,915.20**
Cost of courier service for 8 samples (return)	480.00	
Cost of courier for 96 samples	**5,760.00**	
Total cost of transport for 96 samples	**10,656.00**	**52,915.20**
Building operating cost: maintenance (5%)		
Building operating cost (5%) for 96 samples	**5,102.82**	**5,555.87**
Building operating cost per sample	53.15	57.87
TOTAL RECURRENT COST	**76,996.98**	**148,800.15**
CAPITAL COST		
Buildings		
Total annual cost of building	7,730.02	7,730.02
% used	36.36	54.55
Cost of building for 96 samples	**2,810.92**	**4,216.37**
Equipment		
Annual cost of equipment	**1,879.70**	**1,879.70**
Cost of computer per year	**11.05**	**11.05**
Annual cost of GC	59,612.52	59,612.52
% used for 32 samples	2.27	2.27
Cost per year	**1,354.83**	**1,354.83**
Cost of autosampler (5 years)		42,000.00
Annuity factor (8%, 5 years)		3.99
Annual cost autosampler		10,526.32
% time used		72.73
Cost of autosampler per year		**7,655.50**
Total cost of equipment for 96 samples	**3,245.58**	**10,901.09**
TOTAL CAPITAL COST	**6,056.50**	**15,117.46**
TOTAL COST	**83,053.48**	**163,917.61**
COST PER SAMPLE	**865.14**	**1,707.48**

5.5 DISCUSSION

The cost appraisal found the cost of analyzing two pesticides in a water sample using, ELISA, SPME and SPE to be lower than the cost estimates provided by all local laboratories, with the exception of the State Forensic Laboratory (Table 5.1) which is subsidized (*Senior Analyst pers. comm.*), especially when operating near optimally, and taking into account transport costs and VAT. When accounting for VAT and transport costs (R96.25 per sample, Table 5.6), the cost per sample of SPME and SPE in the study is also substantially lower than the SF laboratory, while the cost per sample of ELISA is similar to that of the SF laboratory. Although most of the surveyed laboratories analyze more than 2 pesticides per sample, their level of detection was not adequate to measure low levels of pesticides of the order of 0.01 µg/L and the only laboratory (ARC) with adequately low levels of detection had a substantially higher cost per sample than found in the study. If more than 50 pesticides were to be analyzed, the estimated cost per sample of SPE and SPME would double, but with the exception of the the SF laboratory, this would still be lower than the other laboratories surveyed.. Due to the high material costs, the cost of analyzing 50 pesticides using ELISA would, however, be too high for routine monitoring.

Factors that could effect the cost per sample are:

- Transport costs, especially high courier costs, which are dependent on distance to the nearest analytical laboratory.
- Administration costs, which were not accounted for in the cost appraisal, but are not thought to be significant.
- The cost of a GC autosampler was not factored into the estimated costs for SPE and SPME. Although it is likely to be used in a laboratory conducting full scale monitoring, this should not have a major impact on the estimated cost per sample for SPE and SPME. The estimated retail price of R80 000 would cancel any cost saving in the technician's laboratory time.
- Training of staff, which was not accounted for, could also have a slight effect on the costs per sample.

The low cost of SPME is particularly important because of the sensitivity and reliability of this method and the faster output compared to SPE. ELISA, although more costly than the other two methods, offers a substantially faster output compared to SPME and SPE. Notably, it is a semi-quantitative method. However, the costs of ELISA will be much higher if more pesticides are analyzed because of the high cost of the plate kits, which were R240 per sample for chlorpyrifos and endosulfan in this project. (i.e. if personnel costs are low for ELISA, there is little room for economy of scale.)

Personnel costs could be reduced if an assistant undertakes sample collection instead of the EHO (estimated cost of EHO's time per sample = R38 for all 3 methods, See Table 5.6). However, the estimated 25% reduction in salary will not significantly impact on the cost per sample, which will decrease by R9.50 for all 3 methods.

Although the time spent on some equipment, especially the ELISA reader, was not estimated, this is not thought to have a significant effect on the cost per sample of the 3 analytical methods.

Although the cost appraisal for integrated sampling was done to compare IM-TWA with IM-AS, it is clear that these methods are presently neither economical nor practical for use in monitoring due to the logistics and costs associated with the collection and analysis of samples. IM-TWA was found to be less costly than IM-AS.

5.6 CONCLUSIONS AND RECOMMENDATIONS

The cost appraisal found that the cost of pesticide analysis by an hypothetical municipal laboratory using one of the 3 analytical methods ELISA, SPME and SPE, was lower than all other unsubsidised local laboratories.

The cost per sample of SPME was found to be the lowest of the three methods and is recommended for the long-term monitoring of pesticide pollution.

The cost of ELISA was the highest, mainly due to the cost of the ELISA plates, but is much faster than the other two methods and might be useful for semi-quantitative monitoring of one or two pesticides in the short-term.

Further work is required to reduce the costs of the 24-hour-TWA-SPME so that it can be affordable for field application.

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Chapter 6

CAPACITY FOR WATER MONITORING IN RURAL AREAS

CONTENTS

6.1 INTRODUCTION

South African water pollution control legislation has changed substantially in recent years. Following a consultative process involving the publication of a White Paper on a National Water Policy for South Africa[1], and a Draft White Paper on Integrated Pollution and Waste Management for South Africa[2], Parliament enacted the National Water Act in 1998 [3]. One of the principal changes contained in the Act is the move toward management of water resources on a catchment basis, based on "demand" rather than supply, and emphasising greater conservation of water resourcesthrough measures such as an appropriate pricing system. The Act also emphasises public participation and provides for greater community involvement in water management structures. Additionally, the DWAF's White Paper recognises the lack of access of millions of South Africans to a safe water supply.

Previous research [4] found consistent pesticide pollution of surface-and groundwater including drinking water in three rural Western Cape agricultural districts. The polluted drinking water was identified mostly in dams and boreholes used by rural communities, but also in two major dams contributing to municipal supplies. Recently, azinphosmethyl and endosulfan resulting from orchard run-off, were also detected in the Lourens River[5]. In a study of indigenous fish species in South African rivers[6], which included the Berg river in the Western Cape, the maximum whole fish load of organochlorines reached high-risk levels in worst-case scenarios (fish eaten daily all year). These studies emphasise the need for pesticide water monitoring in South Africa.

In order to implement monitoring of pesticides in water, it is important to evaluate the capacity that exists and/or is needed to conduct monitoring as well as practices and perception related to pesticide pollution. For this reason a survey of rural local authorities in the Western Cape and other public sector authorities with responsibilities for water management was conducted. The research intended to identify training, technical and logistic support and equipment needed to implement monitoring protocols that include chemical analysis. In addition, similar surveys of farm workers, farmers and rural development organisations (Community-based organisations and NGO's) were intended to ascertain their experience and perceptions.

6.2 AIM AND OBJECTIVES OF THE SURVEY

The aim of the survey was therefore to evaluate the capacity of rural communities to conduct water monitoring

The objectives were:

1. To characterise the knowledge, perceptions and practices of rural target groups regarding pesticide pollution of water.

2. To identify needs for training, technical and logistic support.

6.3 METHODS

A cross-sectional survey of farm workers, environmental health officers (EHO's), local authority management, rural NGO's and government department staff was initially planned. A sample of 12 Western Cape local authorities (L.A) including 8 municipalities and 4 district

councils was randomly selected from a total of 93 obtained from the Western Cape Local Government Organisation. The sample included Ashton, Bonnievale, Bredasdorp, Caledon, Grabouw, Klawer, Oostenberg, and Overberg. The district councils of Plettenberg Bay, Central Karoo, West Coast and Breerivier were also selected.

For every LA it was planned to select 5 officials, 2 NGO staff, 5 farm personnel each from 3 farms for participation in the survey. Separate questionnaires were developed for local authority officials and farm workers by environmental health students from PENTECH supervised by 2 senior researchers from PENTECH and one from UCT (Appendix 6.1 and 6.2). The questionnaire for local authorities included questions on district demographics, current water monitoring on farms in the district and knowledge of pesticides and training received, while the questionnaire for farm personnel included questions on water usage on the farm, water monitoring on farms and knowledge of pesticides and their dangers.

The survey was conducted during September and October 2001 by 9 trained environmental health students from PENTECH supervised by 2 senior researchers from PENTECH and one from UCT. Local authority offices and farms were visited and for logistic reasons were surveyed separately.

6.4 RESULTS

6.4.1 SURVEY OF LA OFFICIALS

Although the project aimed to survey various local officials involved in water monitoring, only 8 EHO from 7 districts could be interviewed (Table 1.1). LA staff other than health personnel were reluctant to participate, perhaps because they viewed water quality as a health matter. As a result, no town clerks, executive officers, or engineers were surveyed.

The districts surveyed included 21 towns with a total population of 503 000.

Table 6.1: Demographic and water monitoring data of districts surveyed

Districts	Towns	Population	Persons testing water(n)	Water sources monitored	Number of times per annum
Boland District Municipality	Robertson, Bonnievale, Ashton	21000	EHO (3)	Municipal sources, canals dams	Variable
Boland District Municipality	Stellenbosch	31000	EHO (2)	Boreholes,dams, rivers	52
Overberg	Caledon	60000	EHO (6)	Boreholes,dams, rivers	43
West Coast	Moreesberg, Malmesbury, Reibeeck West, Darling, Porterville	22000	EHO (5)	Boreholes, canals, roofwater	52
Oostenberg	Brakenfell, Kuilsrivier	79000	EHO (4)	Boreholes, dams	4
Drakenstein	Saron, Wellington, Paarl, Gouda	150000	EHO (14)	Boreholes, dams	75
Breederivier	Rawsonville, Touwsrivier, De Doorns, Worcestor	110000	EHO (NR)	Piped water	43
Witzenberg	Ceres	31000	EHO (2)	Household water	Bacteria:12 Chemicals:4 pH: 365 pesticides:when suspect*

EHO: Environmental Health officer
* The respondent in Ceres when reporting on the number of times water was tested for different quality criteria, said that water is only tested when pollution is suspected.
NR: Not reported

Thus in all seven districts surveyed, water sources on farms are tested by environmental health officers (EHO's). The number of EHO's per district varies according to the population in the area, but it is clear that in some areas such as Oostenberg there are relatively few EHO's for the district population. Only 3 (37%) respondents felt that there were enough persons conducting water monitoring in their area.

Five (71%) respondents felt that their knowledge of pesticides was good, while two (29%) thought it was reasonable.

The water sources being monitored in the areas include municipal sources, canals, dams, boreholes, rivers and roofwater. The typical monitoring procedure is the random collection of a water sample which is then send to a laboratory. The laboratories mentioned were the State Laboratory in Woodstock (SAIMR) the South African Bureau of Standards in Pretoria (SABS), and the Council for Scientific and Industrial Research (CSIR) in Stellenbosch. All the respondents mentioned that water was monitored for bacteria and most (88%) mentioned that chemicals were also monitored but only on request. Only 1 respondent reported that pesticides were monitored, but only when there was a complaint. One respondent also reported that water pH was measured.

All respondents indicated that monitoring was paid for by the municipality, with one stating that the farm owner paid if he or she requested testing.

With regard to feedback of results 1 respondent said that there was feedback to the farmer and the province after every analysis, while 3 said that there was feedback to the farmer on request only.

6.4.2 FARM SURVEY

Sixty-three subjects including 6 sprayers (10%), 35 managers (57%) and 20 (33%) other workers participated in the study.

The subjects were drawn from 16 participating farms from 3 areas including
Boland, West Coast and Overberg. The farms were near 7 towns, which included Bonnievale, Grabouw, Malmesbury, Paardeburg, Riebeeck Kasteel, Riebeeck Rivier and Robertson.

Table 6.2 summarises responses of individuals surveyed and Table 6.3 summarises responses obtained from farms.

Surface water from rivers and dams (used by 44% of farms surveyed), groundwater water from boreholes (used by 25% of farms surveyed), and mountain water (16%), especially in the West Coast, were found to be the most important sources of water in the areas surveyed.

The median distance of participants' homes to the nearest vineyard/orchard on the farm was 400m (range: 5m-10 km).

Sixty eight percent (n=60) reported having taps in the house, while 27 % reported using untapped water.

Farm personnel from 8 (50%) of 16 farms indicated that water was tested on their farms. Testing for chemicals and bacteria was mentioned in all 3 areas surveyed, colour was mentioned in 2 of the areas and pH was mentioned on the one company farm surveyed in Grabouw. No respondent mentioned water being tested for pesticides.

Farm personnel from 3 farms (38%) reported that water was tested by the farmer, 2 (25%) by the municipality, 1 (13%) by a private company, 2 (25%) by the Council of Scientific and Industrial Research (CSIR), and 1 (13%) by a spraying council on the farm.

Table 6.2 Farm survey results: Residents

Variable	Area			
	Boland	West Coast	Overberg	Total
1.Water source and usage	%,n=46	%,n=12	%,n=5	%,n=63
A. Water source:				
Borehole	30	8	0	24
Canal	7	0	0	5
Dam or river	33	33	60	35
Spring	0	8	0	2
Rain	7	0	20	6
Municipal supply	11	8	20	11
Irrigation	2	0	0	2
Mountain	11	42	0	16
B. Water usage:				
Drinking	89	100	80	90
Cooking	83	100	80	86
Irrigation	30	25	20	29
2.Mean distance of home to nearest orchard or vineyard (m), range (n)	400 ;5-10000 (n=44)	600;99-3000(n=11)	20;5-2000 (n=5)	400;5-10000 (n=60)
3.Tapped water	% respondents who said yes			
	59 (n=44)	92 (n=12)	40 (n=5)	64 (n=61)
4.Knowledge of pesticide pollution				
How are pesticides applied?	% respondents who did not know			
	30 (n=44)	0 (n=12)	0(n=3)	13 (n=59)
Can chemicals pollute water?	% respondents who said yes			
	10 (n=41)	20%(n=10)	60 (n=5)	17 (n=54)
5.Knowledge of pesticide health effects				
Can polluted water harm humans?	100 (n=43)	100 (n=12)	100(n=4)	100(n=59)
Chronic effects	% respondents who identified chronic effects			
	8 (n=40)	18 (n=11)	0 (n=5)	9 (n=56)
Health effects	% respondents who did not know health effects			
	23 (n=40)	9 (n=11)	20 (n=5)	20 (56)
6.Training on pesticide health effects	%,n=28	%,n=8	%,n=4	%,n=40
A. Informed or trained about Health effects of pesticides	32	50	0	33
Method of training:				
Farmer's union or co-op	14	13	0	13
Medical person	7	0	25	8
Pesticide or private company	14	25	25	18
Farm management	18	13	50	20
Municipality	4	0	0	3
Brochures	4	0	0	3
Personal studies	7	0	0	5
B. Time of training	%, n=13	%, n=4	%,n=3	%,n=20
< 1 month	0	0	33	5
< 6 month	23	25	67	35
< 1 year	62	0	0	40
C. Follow-up of training	%,n=25	%,n=5	%,n=4	%,n=34
	20	60	50	29

Table 6.3 Farm survey results: Farms

Variable	Area			
	Boland	West Coast	Overberg	Total
1.Water source	%,n=11	%,n=4	%,n=1	%,n=16
Water source:				
Borehole	27	25	0	25
Canal	18	0	0	13
Dam or river	36	50	100	44
Spring	0	25	0	6
Rain	27	0	0	19
Municipal supply	9	25	100	19
Irrigation	9	0	0	6
Mountain	36	75	0	44
2.Water testing	%, n=11	%, n=4	%,n=1	%,n=16
Testing on farm	45	50	100	50
Tested for:	%,n=5	%,n=2	%,n=1	%,n=8
Chemicals	40	25	100	50
Bacteria	20	50	100	50
PH	0	0	100	13
Colour	60	0	100	50
Tested by:				
Municipaity	40	0	0	25
CSIR	40	0	0	25
Farm	20	25	100	38
Spray council on farm	20	0	0	13
Private company	0	25	0	13
Sampling per annum:				
Daily	0	0	100	13
Monthly	40	0	0	25
Yearly	20	25	0	13

With regard to the number of tests done per year: daily monitoring was reported on the 1 company farm in Grabouw, monthly monitoring on 2 farms and yearly monitoring on 2 farms.

Apart from the 1 company farm in Grabouw that does their own water monitoring, only one other farm said that they get feedback on results. Only 2 respondents, both managers, said they have feedback on the results. There were no reports of water run-aways after the test results on any of the farms.

95% of 21 respondents said that they were not trained to test water, and 8% out of 12 managers or owners said that they were.

With regard to knowledge of pesticides, 22% (n=59), all from the Boland area, said that they didn't know how pesticides were applied on the farm. Seventeen percent reported that chemicals could pollute water, while all (n=59) knew that using polluted water could be very harmful and were informed about the possible health effects.

With regard to the effects that can be caused by water polluted by pesticides, 20% (n=56) said that they didn't know, while 9% identified long term effects such as cancer. Forty percent (n=60) said that they were informed about or trained on the adverse effects of using

polluted water, while 45% said they were informed about the relationship between pesticides and health effects.

Out of 40 respondents, 33% said they were not informed or trained with regard to health effects of pesticides, 13% said that they were informed or trained by the farming union or co-op, 8% by a medical person, 18% by a pesticide or private company, 20% by farm management , 3% by the municipality, 3% through brochures and 3% through studies.

With regard to when last training was performed (n=20), 5% said within the last month, 35% said within the last 6 months, 60% said within the last year and 40% said more than a year ago. Ten (29% out of 34) said that there was a follow-up on training.

5% (n=58) reported that they became sick after using polluted water.

6.5 DISCUSSION

The survey was limited by the non-participation of many local authority officials, but valuable information was obtained from environmental health officials who are responsible for water monitoring in their areas and from the farm surveys.

The survey confirmed that there is a lack of water monitoring for pesticides in rural areas in South Africa. Currently, water is mainly being monitored for bacterial contamination, but this varies in different areas from once monthly to once yearly. There is also no effective pesticide monitoring taking place in the areas surveyed. It is therefore clear that water monitoring in many areas needs to be increased.

EHO's are currently responsible for water monitoring in farming areas and they would probably be responsible should water monitoring for pesticides be implemented in future. A previous study [7] evaluating the role of the EHO's in promoting pesticide safety in the Western Cape and evaluating strategies for strengthening their contribution to the public health control of pesticide poisoning, found that their role is limited as they lacked capacity other than conducting inspections. Their role was also found to be reactive in that it depended on notification and was not educational. Although most (70%) of the EHO's that participated in this study felt that their knowledge of pesticide health effects was good, the previous study on EHO's [7] found that their training was theoretical and not practical. They had a limited capacity in providing instruction on pesticide poisonings and pesticide safety issues in general and also had a limited understanding of the circumstances involved in pesticide poisoning. It is also clear that the number of EHO's conducting water monitoring needs to be increased in many areas, and is consistent with previous research findings[7] .
It would appear that the financial responsibility for water monitoring on farms currently lies with the owner or farming co-operative. The survey showed that not all farms have the capacity to monitor water, and it is not clear if those that have existing capacity will be able to monitor pesticides. Most farm personnel that participated in the study are not involved in water monitoring and have not received training nor are they informed about the health effects of pesticides in water.

Currently there appear to be 3 state laboratories that conduct water analysis for pesticides in rural Western Cape areas, with 2 of these situated in Pretoria. These laboratories are not geared to maintain equipment to perform pesticide water analysis with reasonable sensitivity. There is 1 laboratory with sensitive analytical equipment, based in Pretoria, but it is very costly (Chapter 5). If full-scale monitoring of pesticides in water is implemented, the resources and capacity of the current state laboratories will have to be improved substantially or the number of laboratories will have to be increased in order to handle the sample numbers.

The survey also identified that there is little feedback to owners or managers of farms of water monitoring results. This is a serious problem which might deter future participation by communities in monitoring activities.

Water usage results showed that dams (44%) and boreholes (25%) were important sources for domestic use on the farms studied. These findings are consistent with the previous study[5] in the Hex Rriver valley, Grabouw and Slanghoek (usage of boreholes 48% and dams, 52%) and a study in Stellenbosch[a] (usage of boreholes, more than 50% and surface water, 20%). Many participants (27%) in the study also reported using untapped water for domestic purposes. The results therefore confirm that pesticide contamination through domestic use of surface water could potentially be an important route of exposure for farm residents.

The average distance of 400 m from participants' homes to the nearest sprayed vineyards or orchards was much more than in the previous study[5] which found more than 80% homes situated less than 50 m from the nearest sprayed area.

There was a high awareness amongst participants that pesticides are harmful and this is consistent with the previous study[5], but there was a low awareness that pesticides can pollute water (13%) and little knowledge of the health effects of pesticides (55%). There is therefore a need to educate farm residents about the health effects of pesticides, especially with many participants in the study reporting that they were not trained or informed about this. Of the participants who did receive training, more than 40% did not obtain it from the farm, pesticide company or co-op, thus indicating significant opportunities for involvement in training by employers and employer organisations.

6.6 CONCLUSIONS AND RECOMMENDATIONS

There appears to be regular water monitoring in farming areas, but not for pesticides. Monitoring is conducted by EHO's who reported a good knowledge of the health effects of pesticides, but there is a shortage of staff in many areas. There is at present insufficient analytical capacity in the Western Cape to conduct routine pesticide water analysis.

Lack of feedback to owners could be a serious obstacle for future monitoring activities.

The following recommendations are made:

Water monitoring for pesticides needs to be implemented in farming areas and farms. In order to do this, human resources need to be increased and cost-effective screening methods need to be identified. Environmental health officers should be trained in monitoring pesticides. A manual and guidelines need to be fully developed to inform persons conducting water monitoring on the correct procedures to follow in testing water for pesticide contamination. The latter was included in the objectives of this study and is discussed in the next chapter. Existing capacity for conducting pesticide analysis should be enhanced.

Results of any monitoring must be fed back to farmers. A proper management system for handling the data, such as an appropriate database, needs to be developed. Similarly, farm residents need to be informed that pesticides can pollute water and can affect health. Raising awareness could be best done by the farm, farming co-op or pesticide company or could be integrated in current health and safety training mandated by occupational health legislation for farm workers.

[a] Farm survey in the Stellenbosch area: Dopstop project, 1998

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CHAPTER 7:

THE DEVELOPMENT OF GUIDELINES FOR THE PESTICIDE MONITORING OF RURAL WATER

CONTENTS

7.1 INTRODUCTION

The aim of this study was to develop screening methods for monitoring water for pesticides and to provide a guideline document for use by rural communities and/or rural local authorities. There are existing water guideline documents in South Africa but they are aimed at monitoring microbiological, physical and chemical water quality. These documents include a guide, complied by the Department of Water Affairs and Forestry and the Department of Health [1] for health-related assessment of the quality of water supplies. This existing guide summarizes technology and criteria for the monitoring of microbiological, physical and chemical water quality, and in conjunction with the Water Research Commission, a more comprehensive guideline series on the assessment, sampling, analysis, treatment and management of domestic water quality. The first 3 volumes of the latter series on assessment [2], sampling [3] and analysis [4] are now available and the remaining two on treatment and management are not yet available. The International Research Development Centre in Canada has also produced a number of guideline documents on simple and inexpensive tests for detecting chemical and microbiological water pollution for communities in developing countries[5,6].

However, it was not possible for this project to develop such a guideline document, as the field application of the solid phase microextraction fibreholder (Chapter 2) did not reach final assessment. This chapter, however, makes relevant recommendations, and appendix 7.1 provides a potential preamble for a future guideline document.

7.2 FRAMEWORK FOR DEVELOPING GUIDELINES

7.2.1 FACTORS TO CONSIDER WHEN DEVELOPING GUIDELINES

The development of the envisaged guide should take into account factors that would optimise its utility in addition to content.

7.2.1.1 Factors optimizing the utility of a guideline document

It is important that a guideline document has the correct physical structure (size, paper and cover), and is comprehensible to the user. For the former, there should be consultation with a media-training center and for the latter, ,factors such as language and layout are important, and consultation with language experts and institutions involved with the education of target groups would be required. It is expected that environmental health officers would be the most important target group for a guideline document on water monitoring of pesticides and therefore technical colleges involved in their education, should be consulted. It is important for information to be presented in a participative way through one or more workshops with target groups.

It should be pointed out that Appendix 7.1 did not go through the process outlined above.

7.2.1.2 Framework for content areas

Appendix 7.1 in conjunction with findings of the WRC project No.795/1/00 [7], highlights important areas that should be included in a guide on monitoring pesticides in water. This appendix was based on the screening methods developed in Chapters 2-4, the cost associated with these methods detailed in Chapter 5 and the capacity of rural communities to conduct water monitoring, discussed in Chapter 6.

In addition, in order to prevent duplication, information covered in other water guidelines should be taken into account. Consultation with key persons involved in the development of water guidelines should be encouraged. For instance, communication with the Department of Water Affairs and Forestry and Department of Health responsible for current guidelines [1-4] on monitoring water quality should be consulted.

Appendix 7.1 covers areas suggested for inclusion in the proposed guideline document.

Appendix 7.1 sets out a brief background on pesticide pollution in water and refers to levels of contamination and health effects of pesticides contained in WRC project No.795/1/00. [7]

The purpose of the guideline document is discussed, identifying target groups and potential applications, and also outlining the information required before sampling water for pesticide contamination.

Sections include sampling, identification of areas and sites, identification of pesticides to be monitored, procedures to follow and equipment required.

Available laboratory methods are outlined, including the 3 methods investigated by the research team SPE, ELISA and SPME. The relative costs of the 3 methods described in chapter 5, and respective quantification limits and the rapidity of the procedures are discussed.

The last two sections discuss ways to analyse and manage pesticide pollution. The analysis section includes recommendations on the choice of laboratories and how to interpret data and determine if the measured contamination poses human or environmental risks by considering water standards, average daily intakes (ADI) and other criteria. Lists of international water standards and endocrine disruptors are provided as addenda.

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Chapter 8

FULFILLMENT OF STUDY OBJECTIVES

CONTENTS

8.1 STUDY FINDINGS IN RELATION TO PROJECT OBJECTIVES

This project intended to evaluate the usefulness of new sampling and analytical technologies for monitoring of rural water sources. This would strengthen the capacity of rural communities to monitor their water sources.

In terms of the aim of the study set out in Chapter One, most of the main objectives have been met. We have:

1. Evaluated the utility of solid-phase micro-extraction (SPME) fibres and a programmable sampler as tools to achieve integrated sampling of water sources for pesticide pollution (objective 1). The SPME method is a substantial improvement on the traditional SPE method in terms of lower levels of detection (0.01 µg/L), cost (Chapter 5) and turn-around time due to reduced laboratory time. The method was shown to be repeatable. The study was also able to adapt the SPME device to obtain a TWA sample of pesticide polluted water. A theoretical model of the rate of absorption of analyte (pesticide) into the fibre coating, in a particular physical configuration, has been developed and tested using a prototype field device. The prototype requires further development, especially with regard to the loss of analyte during prolonged periods in field and temperature control during in field sampling.

2. Evaluated the utility of enzyme immunoassay kits for the quantitative and semi-quantitative identification of pesticides in water sources tested for pesticide pollution (objective 2). From the tests performed, it appears that the ELISA is semi-quantitative and less time-consuming than the traditional SPE method of analysis, but that reagents, although safe to use, were not as long-lasting.

3. Estimated the costs associated with the SPME and ELISA procedures and compared these to the costs of conventional SPE methods for monitoring pesticides (objective 4). The cost appraisal found SPME to have the lowest cost per sample (R253.86), followed by SPE (R329.86), while ELISA had the highest cost per sample, appreciably higher than SPME. Recurrent costs were high for all three methods, while capital costs for ELISA were low. The cost of supplies for ELISA formed the bulk of the costs (R240 per sample). The costs associated with using the autosampler compared to TWA for obtaining an integrated estimate for 24 hours were almost double (R1707) that of the TWA derived from the SPME fibre (R865), largely because of the capital costs of the autosampler and time required to analyse each sample. However, it is evident that neither of these two methods is presently feasible for routine use in monitoring due to the logistics and costs associated with the collection and analysis of samples.

4. Evaluated the capacity existing in rural communities and local authorities to implement monitoring of water sources for pesticides so as to make recommendations for strengthening such capacity (objective 6). Although many local authority officials generally involved in water monitoring did not participate, valuable information was obtained from farm personnel and environmental health officials who are responsible for water monitoring in their areas. There appears to be regular water monitoring in farming areas, but not for pesticides. This is done by EHO's who reported a good knowledge of the health effects of pesticides, but there is a shortage of staff in many areas. There is not enough analytical capacity in the Western Cape to conduct routine water analysis for pesticides. There is also lack of feedback to owners.

The project was not able to develop a set of guidelines (objective 3) or a manual (objective 5), due to the amount of time taken to develop the analytical tools (objectives 1 and 2), and the fact that these still require further development. These aims have been too ambituous.

Instead, a preamble to a guideline document was developed (Chapter 7 and Appendix 7.1) and methodologies used in the study were summarised (Chapters 2, 3 and 4).

The study therefore found the use of SPME fibres to be an improvement on the traditional SPE method of analysis for organic compounds, specifically for the two pesticides, chlorpyrifos and endosulfan, in water. Further work would be needed to develop integrated methods of sampling. It is unlikely that ELISA analysis could become a useful adjunct in pesticide monitoring due to poor accuracy and high costs, which were much higher than anticipated in the literature.

Rural capacity to monitor pesticides remains limited, although awareness, as demonstrated in this and previous studies, is high. Costs are the chief barrier, and future work to reduce such costs will be the limiting step for any widespread adoption of monitoring. However, the development of tools that give rural communities, local authorities, farmers and farm workers the capacity to initiate, coordinate, understand and interpret monitoring for pesticides in water, are highly desirable.

8.2 RECOMMENDATIONS

The Project Team therefore makes the following recommendations:

THE NEED FOR PESTICIDE WATER MONITORING

1. The DWAF should continue to pursue the development of surveillance and monitoring methodologies to protect water supplies from pollution by pesticides.

2. The DWAF should continue to pursue methods of providing rural communities with simple, cost effective tools to undertake monitoring of their own water supplies.

3. As this project could only develop a preamble to practical guidelines for water monitoring for pesticides, this should be further developed in future for all personnel (community or governmental) charged with inspection and enforcement functions.

IMPROVEMENTS IN SCREENING METHODS

1. At the end of the study, insufficient experimental results had been obtained to provide estimates of the accuracy and sensitivity of the SPME based TWA sampling method. More experimental results and further development work would be needed to overcome the problem of desorption of the analyte during the extended sampling period. Further work would include confirmation of the loss factors to be applied (if valid) to account for desorption of the analyte, evaluation of in-fibre derivitisation (chemically fixing the absorbed analyte) and other techniques to overcome the problem of desorption.

2. There appears to be little advantage to promoting the wider use of ELISA's at this stage mainly due to the cost of suppllies. However, development of in-house plate kits which would substantially reduce costs, could be explored. With reduced costs, ELISA could potentially provide quick semi-quantitative method for screening pesticide water pollution, especially if it can be adapted for use in field.

CAPACITY BUILDING

1. The capacity of state laboratories to measure pesticide water pollution at low levels (0.01 μg/L) using either SPE or SPME methods needs to be improved, especially if routine monitoring is implemented.

2. Behavioural determinants of pesticide pollution should be addressed by encouraging health, safety and environmental training both to employers and employees in farming communities. Any training provided on health and safety should include hazards to the environment amongst the full spectrum of safety information, and be geared to empowerment of rural residents. Particular attention should be given to empowering rural residents to protect themselves and their communities from adverse consequences of pollution.
3. There needs to be feedback to farmers on water results. A proper management system for handling the data, such as an appropriate database, needs to be developed.

4. Farm residents need to be informed that pesticides can pollute water and can affect health. This could be done by the farmer, farming co-op or supplier.

APPENDIX 2.1:

MATERIALS AND METHODS USED FOR SPME

2.1 TABLE OF COMMERCIALLY AVAILABLE SPME FIBRES

Below is a table summarizing the different SPME fibres commercially available[a] for GC and GC/MS.

Phase	Coating Volume	Application
1. Polydimethylsiloxane Three film thicknesses are available: ❖ 7μm ❖ 30μm ❖ 100μm	 0.026μl 0.132μl 0.612μl	Non-polar phase (for many semipolar compounds: aromatics, esters, many pesticides) 100μm used for relatively volatile compounds; the thinner phases are for non-polar and semipolar compounds of low viscosity.
2. 85-μm Polyacrylate	0.521μl	Polar compounds such as phenols, esters.
3. 65-μm Carbowax/ Divinylbenzene	0.357μl	More polar than polyacrylate, for alcohols.
4. 75-μm PDMS/ Divinylbenzene	0.436μl	Moderately polar, for amines.
5. 65-μm Carboxen/ PDMS	0.357μl	Highly volatile compounds including vinyl chlorides, sulphur gases.

a)Supplied by Supelco (supplier's dimensions)

2.2 APPARATUS AND ANALYTICAL METHODS TO BE USED

- Solid phase microextraction fibres - manual sampling and autosampling (Supplier: Supelco; Anatech (South Africa) is the distribution agent)
- Solid phase microextraction fibre holder for manual sampling (Supplier: Supelco; Anatech is the distribution agent)
- Magnetic stirrer (Heidolph Model: MR 3001K) - (Supplier: Labotec); and Teflon coated magnetic stirrer bar
- Millipore Water - Milli-RO4 Water Purification System (Supplier: Millipore), and Milli-Q Reagent Grade Water System (Millipore)
- Gas Chromatograph: Varian 3300
- Varian 8200 CX Autosampler

2.3 FIBRE CARE GUIDELINES (SUPPLIER'S SUGGESTION)

1. A new fibre needs to be conditioned by desorbing for approximately 60 minutes in an injector that is about 10^{o}C hotter than the temperature to be used during analysis. The GC column should be temperature programmed and this procedure should be repeated until there are no major peaks present.
2. The fibre and injector septum should be changed after 100 runs. If, however, after 100 runs the fibre and injector septum are still in a good condition, then further runs with the same fibre may be embarked upon.
3. Standards and samples are prepared and diluted in storage containers and then transferred to manual sampling glass vials for analysis.
   - Samples should be stored in the refrigerator and the glass vials may be chilled before adding the sample.
   - The samples should be transferred to the vials with a pipette of sufficient capacity to deliver the entire sample in one step. (For 10.0ml volume, a 10ml Grade-A pipette should be used).
4. For the liquid sampling, 10.0ml should be used for a 10.0ml glass vial. (Note: The glass vial should not be filled right to the top)
5. The literature suggests that an absorption time of 20-25 minutes is adequate, followed by 3 minutes desorption in the GC port. Preferably, conditions should

be optimized for each analysis. It is not necessary to achieve equilibrium if the total analysis time will be prolonged, because for many samples, relative standard deviations under 5% may be obtained before reaching equilibrium.

2.4 GENERAL RECOMMENDATIONS

The life of the SPME fibre varies with experimental conditions but there is no visible or evident deterioration in chromatography up to 100 runs when desorbing into an injector heated to 220^{o}C. A possible sign of an aging fibre is deterioration of precision, which may also be due to an aging GC septum. Therefore, it is preferable to change the septum when changing the fibre.

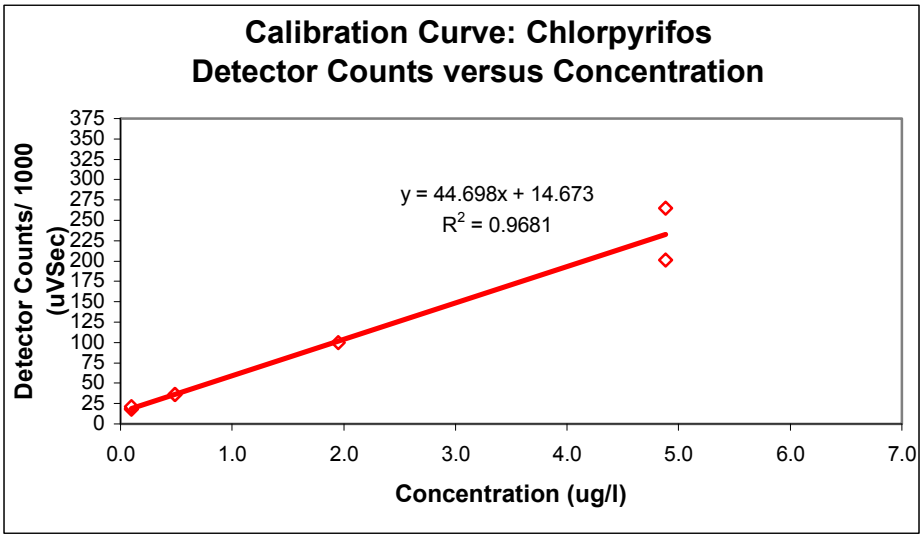
The glass sampling vials should not be filled to the top due to the possibility of carryover if the liquid sample enters the fibre sheath and is not fully desorbed.

Good precision may be obtained without achieving equilibrium. A reasonable sampling (absorption) time is 20-25 minutes, but absorption times may be longer if the GC cycle time permits. At least 3 minutes is recommended to desorb all traces of the analyte to minimize carryover. The injector temperature is normally at least 200^{o}C but should not be higher than the temperature limit of the analytical column or the SPME fibre. (Note: if carryover is present, a longer desorption time and possibly a higher injector temperature should be used as well.)

A new fibre needs to be desorbed for 15 to 20 minutes. When a new fibre is desorbed for 3 minutes, followed by a GC run, after 6 runs the blank is clean. For a fibre that has been used, the first run each day should be a blank. Peaks known as Ghost peaks may occasionally appear from the vial septa. If this occurs, a different septum brand should be used or the septa should be baked before use.

2.5 CALIBRATION CURVES

Figure 1: Calibration Curve of Chlorpyrifos – Detector Counts versus Concentration*



**All Calibration Curves were generated using Microsoft Excel, and fitted with a linear trendline.*

Figure 2: Calibration Curve of Endosulphan Sulfate - Detector Counts versus Concentration

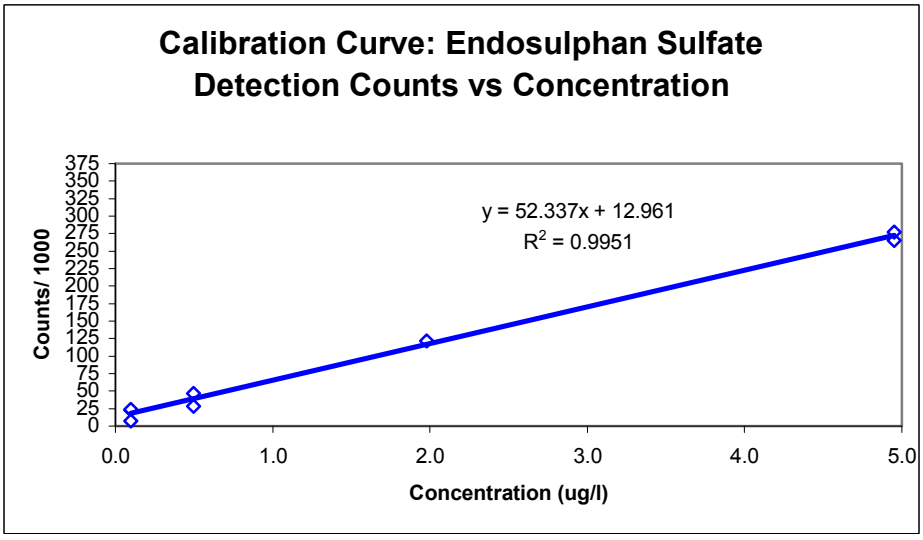


Figure 3: Calibration Curve of Endosulphan-beta - Detector Counts versus Concentration

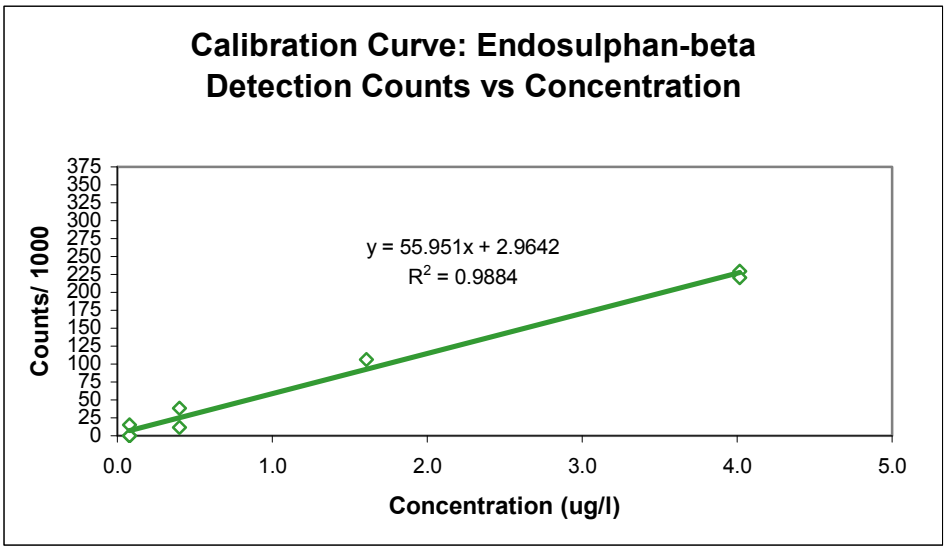
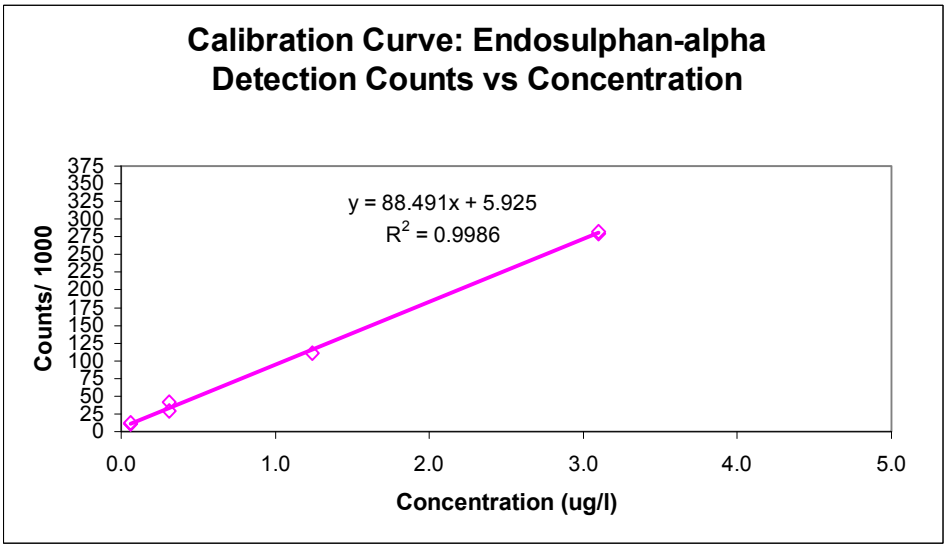


Figure 4: Calibration Curve of Endosulphan-alpha - Detector Counts versus Concentration



APPENDIX 4.1&4.2:

ILLUSTRATION OUTLINING THE ELISA ASSAY USING A RAPID ASSAY AS AN EXAMPLE

Appendix 4.1 Ilustration outlining the principal of operation of the ELISA assay using a rapid assay as an example

Technical Concept
Enzyme linked immunosorbent assays (ELISA's) combine selective antibodies with sensitive enzyme reactions to produce analytical systems capable of detecting very low levels of chemicals. Ohmicron's technical concept is based on the use of novel magnetic particles as the solid support and means of separation in an ELISA system.

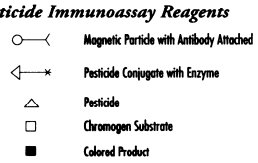
Enzyme Immunoassay Chemistry
The immunochemical reaction contributes high selectivity, due to the extraordinary discriminatory capability of antibodies, and high sensitivity, because of the powerful catalytic capability of enzymes.

Magnetic Particles
Ohmicron utilizes novel magnetic particles to overcome problems common to conventional immunoassay procedures. A variety of separation approaches have been applied to immunoassays, including coated tubes, particulate systems, and double antibody separation methods. Coated tubes and coated microtiter plates allow separation of bound and free label without centrifugation, but they have three major disadvantages. First, the surface of the tube/plate limits the amount of antibody that can be used in the reaction. Second, the antibody is far removed from some of the antigen, slowing the reaction between the antibody and the antigen. Finally, the antibody is absorbed, not covalently bound, to the solid phase, which may contribute to loss of precision. Particulate systems frequently settle during assays, and must be centrifuged to separate bound from free label. Double antibody methods require an additional incubation period for the second antibody, and still require centrifugation to pellet the antibodies.

The unique properties of the one micron sized magnetic particles as solid support for the RaPID Assay solves these problems. Covalent bounding of antibodies to magnetic particles eliminates antibody dissociation that might cause imprecision. Their size allows for even dispersion throughout the reaction mixture. This places the antibody in proximity with other reactants creating rapid reaction kinetics. In addition, they contribute the means for separating bound from free enzyme, enabling these systems to be very simple and easy to use.

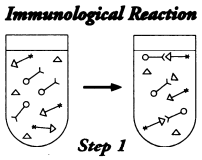
Assay Steps
The use of the system for detection and quantitation of a pesticide in a sample is illustrated in Figures 1 – 3.

Legend



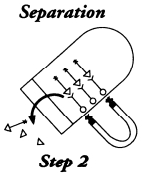
In step one, the sample to be analyzed is added, along with the pesticide labeled with an enzyme, to a disposable test tube containing antibodies attached to magnetic particles. Any pesticide present in the sample competes with the labeled pesticide for binding sites on the antibodies. This immunological reaction occurs for 15 to 30 minutes (Figure 1).

Figure 1



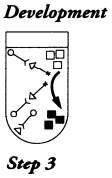
In step two, a magnetic field is applied to the magnetic particles with pesticide and labeled pesticide bound to the antibodies. All particles are pulled and held to the tube wall while excess reagents are decanted. The particles are washed twice (Figure 2).

Figure 2

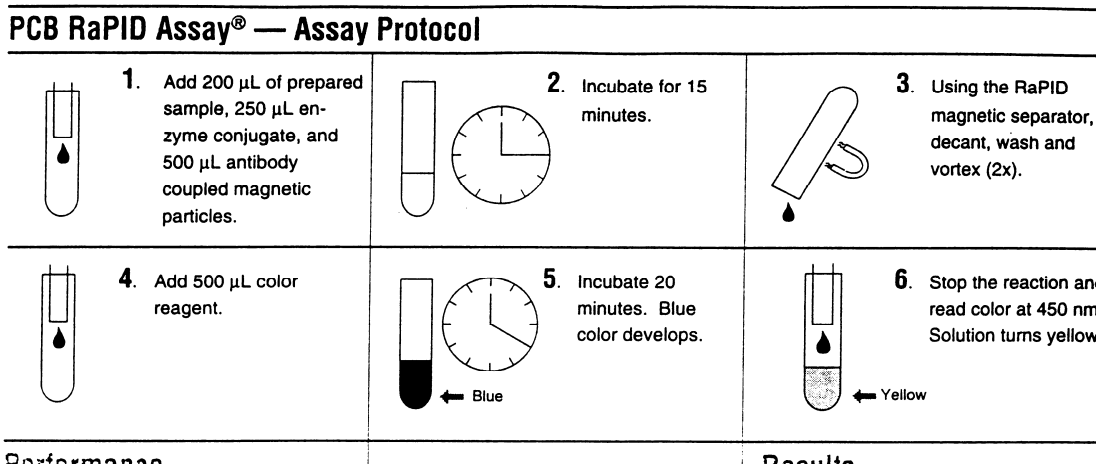


In step three, the amount of enzyme labeled pesticide is determined by adding hydrogen peroxide, and chromogen, generating a colored product (Figure 3). After a short incubation, the color production is stopped and stabilized by the addition of acid. Since the labeled pesticide was in competition with pesticide in the sample for the antibody sites, the color development is proportional to the enzyme labeled pesticide and inversely proportional the concentration of pesticide in the sample.

Figure 3



Appendix 4.2 Ilustration outlining the procedure of performing an ELISA assay using the PCB rapid assay as an example



APPENDIX 5.1&5.2:

SUMMARY OF THE COSTS OF ANALYTICAL METHODS AND SAMPLING FOR MEASURING PESTICIDE WATER POLLUTION

Appendix 5.1 Summary of the costs of 3 analytical methods, ELISA, SPE and SPME for measuring pesticide water pollution

Cost category/method of analysis	ELISA	SPME	SPE
RECURRENT COST			
Personnel cost (salary)			
lab technician monthly	10,000.00	10,000.00	10,000.00
% time spent on 32 samples	1.70	18.18	36.36
cost of time per month (32 samples)	170.45	1,818.18	3,636.36
cost of time per year (384 samples)	**2,045.45**	**21,818.18**	**43,636.36**
EHO monthly	6,666.66	6,666.66	6,666.66
% time spent collecting 32 samples	18.18	18.18	18.18
cost of time per month (32 samples)	1,212.12	1,212.12	1,212.12
cost of time per year (384 samples)	**14,545.44**	**14,545.44**	**14,545.44**
Total personnel cost for 384 samples	**16,590.89**	**36,363.62**	**58,181.80**
Supplies			
cost of 2 *fibres* (180 wells)	11,000.00		
cost per well	61.11		
number of wells per 8 samples	32.00		
cost of wells for 8 samples	1,955.56		
cost of fibre per sample	244.44		
cost of fibre for 384 samples	**93,866.67**		
cost of a SPME *fibre*		1,000.00	
cost of fibre per sample		10.00	
cost of a fibre for 384 samples		**3,840.00**	
cost of *chlorpyrifos standard*		2,000.00	2,000.00
cost of chlorpyrifos standard per sample		2.00	2.00
cost of ch. standard for 384 samples		**768.00**	**768.00**
cost of *endosulfan standard*		3,912.00	3,912.00
cost of endosulfan standard per sample		3.91	3.91
cost of end. standard for 384 samples		**1,502.21**	**1,502.21**
cost of *methanol*		300.00	300.00
cost of methanol per sample		0.30	0.30
cost of methanol for 384 samples		**115.20**	**115.20**
cost of *nitrogen gas*		1,500.00	1,500.00
cost of nitrogen gas for 384 samples		**1,500.00**	**1,500.00**
cost of *carbon cartriges*			1,300.00
cost of carbon cartridge per sample			26.00
cost of carbon cartridge for 384 samples			**9,984.00**
Total cost of supplies for 384 samples	**93,866.67**	**7,725.41**	**13,869.41**
Transport cost			
number of km travelled per month (32 samples)	80	80	80
number of km travelled per sample	2.5	2.5	2.5
fixed running cost per km (AA rate)	1.71	1.71	1.71
petrol and maintenance per km (AA rate)	0.84	0.84	0.84
cost of transport per sample	6.38	6.38	6.38
cost of transport for 384 samples	**2,448.00**	**2,448.00**	**2,448.00**
cost of courier service for 8 samples	770.00	770.00	770.00

APPENDIX 5.1&5.2:
SUMMARY OF THE COSTS OF ANALYTICAL METHODS AND SAMPLING FOR MEASURING PESTICIDE WATER POLLUTION

cost of courier service per sample	96.25	96.25	96.25
cost of courier for 384 samples	**36,960.00**	**36,960.00**	**36,960.00**
Total cost of transport for 384 samples	**39,408.00**	**39,408.00**	**39,408.00**
Building operating cost: maintenance (5%)			
cost of building for 384 samples	90.34	1,405.46	2,810.92
cost of equipment for 384 samples	8,365.02	7,343.24	7,098.87
total cost for 384 samples	8,455.36	8,748.69	9,909.79
electricity and water per year	4,800.00	4,800.00	4,800.00
Building opertaing cost (5%) for 384 samples	**5,222.77**	**5,237.43**	**5,295.49**
building operating cost per sample	13.60	13.64	13.79
TOTAL RECURRENT COST	**155,088.33**	**88,734.46**	**116,754.70**
CAPITAL COST			
Buildings			
cost per square meter (office)	2,736.00	2,736.00	2,736.00
cost per square meter (lab)	3,232.00	3,232.00	3,232.00
size of office	10.00	20.00	20.00
size of lab	10.00	10.00	10.00
cost of office	27,360.00	54,720.00	54,720.00
cost of lab	32,320.00	32,320.00	32,320.00
annuity factor (8%, 30 years)	11.26	11.26	11.26
annual cost of office	2,429.84	4,859.68	4,859.68
annual cost of lab	2,870.34	2,870.34	2,870.34
total annual cost of building	5,300.18	7,730.02	7,730.02
% used	1.70	18.18	36.36
Cost of building for 384 samples	**90.34**	**1,405.46**	**2,810.92**
cost per sample	0.24	3.66	7.32
Equipment			
cost of equipment (5 years)	33,200.00	7,500.00	6,525.00
annuity factor (8%, 5 years)	3.99	3.99	3.99
annual cost of equipment	**8,320.80**	**1,879.70**	**1,635.34**
cost for (8x4x12) = 384 samples	21.67	4.90	4.26
cost of computer (3 years)	10,000.00	10,000.00	10,000.00
annuity factor (8%, 3 years)	2.57	2.57	2.57
annual cost of computer	3,891.05	3,891.05	3,891.05
% used for 32 samples	1.14	1.14	1.14
cost of computer per year	**44.22**	**44.22**	**44.22**
cost of computer per 1 sample	0.12	0.12	0.12
cost of GC (10 years)		400,000.00	400,000.00
annuity factor (8%, 10 years)		6.71	6.71
annual cost of GC		59,612.52	59,612.52
% used for 32 samples		9.09	9.09
cost per year		**5,419.32**	**5,419.32**
cost per sample		14.11	14.11
Total cost of equipment for 384 samples	**8,365.02**	**7,343.24**	**7,098.87**
TOTAL CAPITAL COST	**8,455.36**	**8,748.69**	**9,909.79**
TOTAL COST	**163,543.69**	**97,483.16**	**126,664.49**
COST PER SAMPLE	**425.90**	**253.86**	**329.86**

Appendix 5.2 Summary of the costs of two integrated methods of sampling, 24-hour-TWA and 24-hour-autosampling for measuring pesticide water pollution

Cost category/method of analysis	TWA	autosampler
RECURRENT COST		
Personnel cost (salary)		
lab technician monthly	10,000.00	10,000.00
% time spent on 8 samples	36.36	72.73
cost of time per month (8 samples)	3,636.36	7,272.73
cost of time per year (96 samples)	**43,636.36**	**87,272.73**
EHO monthly	6,666.66	
% time spent collecting 8 samples	18.18	
cost of time per month (8 samples)	1,212.12	
cost of time per year (96 samples)	**14,545.44**	
Total personnel cost for 96 samples	**58,181.80**	**87,272.73**
Supplies		
cost of a SPME *fibre*	1,000.00	1,000.00
cost of fibre per sample	10.00	10.00
cost of a fibre for 96 samples	**960.00**	**960.00**
cost of *chlorpyrifos standard*	2,000.00	2,000.00
cost of chlorpyrifos standard per sample	2.00	2.00
cost of ch. standard for 96 samples	**192.00**	**192.00**
cost of *endosulfan standard*	3,912.00	3,912.00
cost of endosulfan standard per sample	3.91	3.91
cost of end. standard for 96 samples	**375.55**	**375.55**
cost of *methanol*	300.00	300.00
cost of methanol per sample	0.30	0.30
cost of methanol for 96 samples	**28.80**	**28.80**
cost of *nitrogen gas*	1,500.00	1,500.00
cost of nitrogen gas for 96 samples	**1,500.00**	**1,500.00**
Total cost of supplies for 96 samples	**3,056.35**	**3,056.35**
Transport cost		
cost of car hire for 16 days		3,200.00
number of km travelled per month (8 samples)	160.00	3,840.00
petrol and maintenance per km (AA rate)	2.55	0.84
petrol and maintenance per month	408.00	1,209.60
cost of transport per month	408.00	4,409.60
cost of transport for 96 samples	**4,896.00**	**52,915.20**
cost of courier service for 8 samples	30.00	
cost of courier service for 8 samples (return)	480.00	
cost of courier for 96 samples	**5,760.00**	
Total cost of transport for 96 samples	**10,656.00**	**52,915.20**
Building operating cost: maintenance (5%)		
cost of building for 96 samples	2,810.92	4,216.37
cost of equipment for 96 samples	3,245.58	10,901.09

APPENDIX 5.1&5.2:
SUMMARY OF THE COSTS OF ANALYTICAL METHODS AND SAMPLING FOR MEASURING PESTICIDE WATER POLLUTION

total cost for 96 samples	6,056.50	15,117.46
electricity and water per year	4,800.00	4,800.00
Building opertaing cost (5%) for 96 samples	**5,102.82**	**5,555.87**
building operating cost per sample	53.15	57.87
TOTAL RECURRENT COST	**76,996.98**	**148,800.15**
CAPITAL COST		
Buildings		
cost per square meter (office)	2,736.00	2,736.00
cost per square meter (lab)	3,232.00	3,232.00
size of office	20.00	20.00
size of lab	10.00	10.00
cost of office	54,720.00	54,720.00
cost of lab	32,320.00	32,320.00
annuity factor (8%, 30 years)	11.26	11.26
annual cost of office	4,859.68	4,859.68
annual cost of lab	2,870.34	2,870.34
total annual cost of building	7,730.02	7,730.02
% used	36.36	54.55
Cost of building for 96 samples	**2,810.92**	**4,216.37**
cost per sample	29.28	43.92
Equipment		
cost of equipment (5 years)	7,500.00	7,500.00
annuity factor (8%, 5 years)	3.99	3.99
annual cost of equipment	**1,879.70**	**1,879.70**
cost for (8x12) = 96 samples	19.58	19.58
cost of computer (3 years)	10,000.00	10,000.00
annuity factor (8%, 3 years)	2.57	2.57
annual cost of computer	3,891.05	3,891.05
% used for samples	0.28	0.28
cost of computer per year	**11.05**	**11.05**
cost of computer per 1 sample	0.12	0.12
cost of GC (10 years)	400,000.00	400,000.00
annuity factor (8%, 10 years)	6.71	6.71
annual cost of GC	59,612.52	59,612.52
% used for 32 samples	2.27	2.27
cost per year	**1,354.83**	**1,354.83**
cost per sample	14.11	14.11
cost of autosampler (5 years)		42,000.00
annuity factor (8%, 5 years)		3.99
annual cost autosampler		10,526.32
% time used		72.73
cost of autosampler per year		**7,655.50**
Total cost of equipment for 96 samples	**3,245.58**	**10,901.09**
TOTAL CAPITAL COST	**6,056.50**	**15,117.46**
TOTAL COST	**83,053.48**	**163,917.61**
COST PER SAMPLE	**865.14**	**1,707.48**

APPENDIX 5.1&5.2: SUMMARY OF THE COSTS OF ANALYTICAL METHODS AND SAMPLING FOR MEASURING PESTICIDE WATER POLLUTION

APPENDIX 6.1 & 6.2:

SURVEY QUESTIONNAIRE (FARM PERSONNEL AND LOCAL AUTHORITIES

Appendix 6.1a:

QUESTIONNAIRE USED FOR SURVEY OF FARM PERSONNEL

Afrikaans version

ONDERSOEK VAN WATER BESMET DEUR INSEKDODERS/GIFSTOWWE (Plaaswerkers)

Streek/Gebied:
Plaasnaam/Opstal:
Naam:
Neming/Amp:
Datum:
Onderhoud Voeder:

1. Waarvandaan kry u water?
   Rivier/ Stroom/ reenwater/ dam/ boorgat/ munisipale kraan/ oog

2. Hoe ver is die water vanaf die boord ?

3. Hoe kry jy die water tot (hier) daar.
   Emmers/pyp/kanne plastic of metal en ander.

4. Hoe bêre jy die water tuis?

5. As kanne gebruik word waar kry of koop jy die kanne?
   Koop by opstal, winkel, van die boer/ou chemise houers.

6. Hoe maak u die kanne skoon?

7. Waarvoor gebruik u die water?
   Om te drink/ koskook/ besproeing/was, klere of u self, swem.

TOETS VAN WATER

8. Is die water vooraf getoets?/Indien ja hoe word dit getoets?

Proer	
Bakteriologies	
Kleur	
Chemikalies opgeloste gifstowwe	

9. Indien ja, waarvoor toes jy/julle die water

10. Wie doen die toetse?

11. Hoe gereeld word die H2O getoets?

12. Wat maak julle met die toets uitslae?

13. Kry julle die toets resultate terug indien ja wat doen julle met die resultate?

14. Het u ooit die water laat wegloop na die uitslae

15. Wat word gebruik om die water te toets?

16. Is u opgelei om die water te toets?

17. Wie betaal vir die toets, of die aankope om die toets te doen?

18. Is ander mense, ingelig omtrent die uitslae van die toetse?

KENNIS VAN GIFSTOF- BESOEDELING

19. Hoe word gifstowwe op die plaas toegepas?

20. Weet u wat besmet die water?

21. Wat kan gebeur as u besmette water gebruik?

22. Weet u die effekte wat water wat besoedel is met gifstowwe op die liggaam kan hê

23. Is u ingelig en / of opgelei omtrent die nuwe effekte van te gebruik?

24. Het iemand siek geword nadat hulle besmette H2O gedrink het?

25. Is u ingelig oor die vervantskap tussen gifstowwe en die nuwe effekte?

26. Deur wie is hulle onderrig of ingelig?

27. Hoe lank gelede was die opleiding uitgevoer?

28. Is daar enige opvolg kurses aangebied?

Appendix 6.1b:

QUESTIONNAIRE USED FOR SURVEY OF FARM PERSONNEL

English version

STUDY OF WATER CONTAMINATED BY INSECTICIDES/PESTICIDES ()

Region/Area:
Farmname:
Name:
Jobtitle:
Date:
Interviewer:

1. Where do you obtain water from?
   River// Stream/ rainwater/ dam/ borehole/ municipal tap

2. How far is the water source from the orchad/vineyard?

3. How do you carry the water to there (here)
   Bucket/pipe/containers plastic or metal or other.

4. How do you store the water at home?

5. If you use containers, where do you obtain or buy the containers?
   Buy at shop, store, from farmer, old containers.

6. How do you clean the containers?

7. For what purpose do you use the water?
   To drink/ cook food/ irrigation/wash, clothes or yourself, swim.

TESTING OF WATER

8. Does the water get tested before use?/If yes, how?

Taste	
Bacteria	
Colour	
Chemically dissolved, pesticides	

9. If yes, for what do you test the water?

10. Who does the testing?

11. How regularly does the water get tested?

12. What do you do with the test results?

13. Do you get the test results back, if yes, what do you do with the test results?

14. Did you ever had the water divert away due to the results?

15. What is used to test the water?

16. Are you trained to test water?

17. Who pays for testing, or to have testing performed?

18. Are others informed about the results of the tests?

KNOWLEDGE OF CHEMICAL POLLUTION

19. How are pesticides applied on farms?

20. Do you know what can pollute water?

21. What can happen if you use polluted water?

22. Do you know what the effects are of pesticide polluted water on the body?

23. Are you informed and / or trained about the adverse effects of using polluted water?

24. Do you know of someone that became ill after drinking polluted water?

25. Are you informed about the relationship between pesticides and adverse effects?

26. Who trained or informed you?

27. How long ago was then training performed?

28. Were there any follow-up courses offered?

Appendix 6.2a:

QUESTIONNAIRE USED FOR SURVEY OF LOCAL AUTHORITY OFFICIALS

Afrikaans version

MONITEERING VAN WATER BRONNE (Plaaslike Bestuur)
Datum:
Area/Kontrei/Streek:
Naam van plaastike bestuur:
Naam van persoon met wie onderhoud gevoer is:
Titel van persoon met wie onderhoud gevoer is?
Totale bevolking

1. Wat is u kennis van gifstowwe?

2. Watter bronne van H2O word getoets?

3. Wie is verantwoordelik vir die toets van water in u munisipaliteit?

4. Hoeveel persone is verantwoordelik vir die toets van water?

5. Is daar genoeg personeel om al die H2O op ‘n gereelde basis te toets?

6. Is die persone wat die water toets enigsins opgelei?

7. Wie behartig die opleiding?

8. Watter apparate word gebruik om H2O te toets?

9. Hoe word H2O getoets?

10. Waar word water geanaliseer?

11. Hoe gereeld word die water per jaar getoets?

12. Waarvoor word die H2O getoets?
    Kieme/ chemikalie/ skoonheid/ gifstowwe/ ander

13. Wie betaal vir die analise van H2O in die munisipaiteit?

14. Hoe gereeld word die informasie van die toetse versprei na boere, gemeenskap gesondheidwerkers regering?

Appendix 6.2b:

Questionnaire used for survey of local authority officials

English version

MONITORING OF WATER SOURCES (Local Athority)
Date:
Area/Region/District:
Name of Local Athority:
Name of person interviewed:
Title of person interviewed:
Total population

1. What is your knowledge of pesticides?

2. Which sources of water is tested?

3. Who is responsible for testing water in your municipality?

4. How many persons are responsible for testing water?

5. Are there enough persons to test the water regularly?

6. Are the persons testing the water trained?

7. Who trains them?

8. Which instrument is used to test water?

9. How is water tested?

10. Where is the water analysed?

11. How many times per annum does the water get tested?

12. What does the water get tested for?
    Germs/ chemicals/ cleanliness/ pesticides/ other

13. Who pays for the analyses of water in the municipality?

14. How regularly is the information about the tests sent to farmers, community, health workers or the government?

APPENDIX 7.1:

GUIDELINES FOR THE MONITORING OF PESTICIDES IN WATER

BACKGROUND

Environmental sustainability is important for future economic growth, and water pollution by pesticides affects many biological systems. Contamination of groundwater is particularly important because it can take a long time to recover.

Pesticides are widely used in South Africa, predominantly in agricultural applications but also in vector control in public health, commercial pest control, domestic use and in selected industrial or food processing technologies. Such sources of pesticide exposure can result in contamination of water sources, through routes that are difficult to predict.

For example many South African farm workers live on the farms where they work, in close proximity to the fields or orchards that are subject to pesticide application[1], often by aerial methods. Domestic exposure to humans may arise from environmental contamination through polluted water sources, particular if surface water is used for drinking or other domestic purposes. Similarly, inappropriate or careless use of pesticides for vector control, control of alien vegetation or other seemingly important public purposes, may result in water pollution by pesticides.

A review of factors contributing to the contamination of water by pesticides, actual levels of contamination, and possible health effects, is provided in WRC Report No. 795/1/00.[2]

PURPOSE OF GUIDE

The purpose of this guide is to provide information on the correct procedure that should be followed when collecting water samples.

WHO CAN MAKE USE OF THE GUIDE?

* Monitoring and enforcement authorities (e.g. EHOs, Health Inspectors), NGOs, Agricultural Sector (farmers and unions). The guide offers practical methods for monitoring pesticide pollution so as to comply with standards, to identify the need for remediation, and to achieve water of the desired quality.
* Water supply agencies – for the determination of a safe water supply.
* Water resource developers – for assessing whether a raw water source is suitable for supply
* Educators - for promoting an understanding of the importance of correct water sampling techniques.
* The public - for information on correct procedure to follow when collecting samples for analysis to determine the quality of their water supply.

WHY IS IT IMPORTANT TO KNOW HOW TO COLLECT WATER SAMPLES?

Wrong sampling procedures and methods will affect the accuracy and reliability of analytical results and lead to misleading conclusions on the quality of the water supply.

WHAT DO I NEED TO KNOW BEFORE SAMPLING FOR WATER POLLUTION BY PESTICIDES?

Before collecting a water sample to investigate pesticide water pollution, one needs to identify pollution areas and sampling points; to know when and how many times to collect samples and which pesticides to measure.

HOW DO I IDENTIFY AREAS AND SAMPLING POINTS?

Table 1 outlines important criteria needed to identify study areas for pesticide water pollution; key factors with regard to these criteria and sources of information.

Table 1: Criteria used for choice of study areas - factors associated with highest likelihood of pesticide pollution of water

CRITERION	KEY FACTORS	SOURCE
Annual rainfall	Run-off to surface water and leaching into ground water are initiated by rainfall triggers	Department of Water Affairs rainfall maps and 1997-1998 rainfall data from the SA Weather Bureau
Water table	A shallow water table (< 5 m) favours groundwater pollution	Consultation with geohydrologist
Soil characteristics	Upper soil characteristics governing run-off and leaching, includes permeability (soil structure and texture), organic matter content, moisture, pH and cation exchange capacity.	Direct measurements absent. Soil mapping data, ARC Institute for Soil, Water and Climate (Stellenbosch), interpreted by soil scientists from Elsenburg Agricultural College
Intensity of agriculture	Intensity and duration of pesticide spraying	Sales data, which are costly. Consultations with persons in industry
Accessibility of areas.	Areas where accessibility could be arranged.	Farming unions, Environmental Officers and Co-ops.
Boreholes	Groundwater usage	Borehole data from the Department of Water Affairs

Sampling points in each area should be selected in order to capture a spectrum of ground and surface water points and in configurations that could assist in explaining potential routes and sources of contamination.

It is important to also include water used for drinking purposes.

Sites can include points along a river potentially contaminated with pesticides; farm and water storage dams; sub-surface and surface farm drains; farm water reservoirs; wells; boreholes and taps.

WHICH PESTICIDES SHOULD I MONITOR?

Due to prohibitive costs, not all pesticides can be measured, and the most important ones should be selected. To decide on which pesticides to target, expert industry spraying programmes can be used to draw up an initial list. From this list, pesticides can be selected on the basis of:

- Their potential to cause adverse health impacts. This can be assessed by looking at the drinking and aquatic health standard set locally or internationally,[3,4] Average Daily Intakes (ADI) set by the WHO,[5] as well other health-effect indicators such as toxicological data, potential to cause cancer, and endocrine disruption potential. For the latter there are various lists available.[6-8]
- Usage of pesticides which can be determined from industry and from the literature.[9,10] Usage data can also be obtained from farmers, commercial spray operators and cooperatives
- The likelihood of contamination by the pesticide which can be based on a combination of industry expert opinion, presence on the EPA list of water pollutants,[11] and known groundwater ubiquity scores,[12] which are developed to screen leachability of pesticides by means of organic sorption coefficient and half-life in soil.

The final list of pesticides selected for analyses will be conditioned by the local availability of methods for analysis.

There can be initial screening of pesticides by a laboratory equipped to perform bulk analyses.

FIELD SAMPLING

Sampling will be done using grab sampling, which involves collection of the sample in a container, which is then sent to a laboratory for analysis. The limitation of grab sampling is that it measures contamination at one point in time and does not give an accurate average estimate of fluctuations in pesticide levels occurring during the day. Direct field testing and integrated sampling methods measuring pesticide levels over the whole day in the field and thereby providing an average estimate of contamination during the day, are currently not practical.

SAMPLING EQUIPMENT FOR GRAB SAMPLES

1. Sample bottle – amber glass, 1l or bigger fitted with screw cap lined with Teflon or clean aluminium foil. If amber bottles are not available, protect sample from light.
2. The bottles must be washed with water, rinsed with acetone or methylene chloride or methanol and air dried to minimize contamination.

GRAB SAMPLE COLLECTION, PRESERVATION AND HANDLING

1. Grab samples must be collected directly into the bottle without rinsing it.

2. Samples should preferably be chilled to 4°C or below from time of collection until extraction.

3. It is recommended that at least 3L of water be collected to represent one sample from one water source.

4. A sample collection report needs to be filled in every time a set of samples is collected.

5. Sampling bottles must be clearly labelled showing sample number, date and name of sample collector.

6. Samples must be Isent immediately for analysis.

Samples should be taken at least once weekly and twice in the week after the first rainfall trigger (>10 mm over 24 hours or >15 mm over 48 hours), using a standardized collection procedure.

HOW DO I CHOOSE A LABORATORY FOR ANALYSIS?

The following criteria should be considered when sending a water sample to a laboratory for pesticide analysis.

1. Analysis must be conducted by a laboratory accredited for Good Laboratory Practice

2. The laboratory should be properly equipped, have the necessary facilities and staff competence to undertake the analysis.

3. It is important that the quantification limits of the laboratory are adequately sensitive for the pesticides being measured. It is important to measure pesticides in the parts per billion range as these are the levels at which water standards are set.

4. The laboratory should have an adequate quality control and quality assurance programme whereby results are confirmed in the laboratory and with another laboratory. It is therefore recommended that analysis be performed wherever possible by accredited laboratories, because accreditation guarantees appropriate standards of laboratory organisation and of QA/QC.

5. The laboratory should manage data systematically and make it easily accessible. The sheer volume of data accumulated after just a few years of monitoring dictates the adoption of computer-based data management systems as the basis for data storage and management. The choice of a particular database depends on the types and intended use of the data and on compatibility, both of the computer hardware and software and with other databases.

6. The analyses should be fully documented. The ability to trace analytical results from the laboratory report back to the original sample is an essential component of good laboratory practice, and is a prerequisite for accreditation of analytical laboratories. Apart from its chain of custody details for each sample, the laboratory record system must include the following information for each analysis:

    - identity of the sample analysed;
    - identity of analyst;
    - name of equipment used;
    - original data and calculations;
    - identification of manual data transfers;
    - documentation of standards preparation;
    - use of certified calibration solutions.

7. The accuracy of the analytical methods, bias and precision must be established.

WHICH METHODS ARE AVAILABLE TO DO PESTICIDE ANALYSES?

It is important to note which methods are used by the laboratory to analyse the water samples. The methods available are :

1. Traditional Solid Phase Extraction using Gas Chromatography. The time period to obtain results could be long, but they are accurate, cost-effective and readily available. The detection limits are in parts per billion.

2. Solid Phase Micro Extraction (SPME) is a technique whereby pesticides are adsorbed from water onto a coated silica fibre. The fibre is then analysed using Gas Chromatrography (GC). Such methods are cost-effective, quick, and accurate. The detection limits are in parts per billion.

3. Enzyme-linked immunoassays (ELISAs) are semi-quantitative tests. They are capable of detecting low levels of chemicals with high specificity and are quicker than SPE and SPME methods. The disadvantages are the prohibitive costs. The detection limits are an order of magnitude (10 times) more than for SPE and SPME methods.

HOW TO INTEPRET THE LABORATORY DATA

When interpreting the results it is important to determine if there is consistent contamination, if there is contamination of drinking water and if the levels of contamination are of concern to human health and the environment. With respect to health and the environment, it is

important to see if detections regularly exceed regulatory drinking and aquatic water standards for pesticides (summarised in Addendum A). South Africa does not have drinking water standards for pesticides, but does have standards for aquatic systems. There are, however, a number of international drinking water standards including the WHO Drinking Water Guidelines, the USEPA Drinking Water Standards and Health Advisories and European Union Drinking Water Standards. It is always useful to first determine the number of detections exceeding the Standard set by the European Union which is universal for all pesticides and is the lowest of all the standards (0.1 parts per billion for all pesticides, 0.5 for a combination of pesticides), before determining if levels exceed higher water standards, **if these are available for the pesticides being measured.**

It is also important to determine if average daily consumption of contaminated drinking water exceeds the average daily intake (ADI) levels set by the WHO[5]. This can be done by:

i) using the mean and peak levels of contamination
ii) assuming an average daily water intake of 2L and a body weight of 60kg
iii) calculating the root mean square of the average daily intake
iv) calculating what percentage this forms of WHO ADI's.[5]

The WHO uses a range of 1-10%, that must be contributed by pesticides to drinking water.

Other methods for determining if pesticide contamination is of concern to health are by referring to WHO toxicological and carcinogenicity classifications, as well as whether a particular pesticide is a known endocrine disruptor (Addendum B).

If levels of contamination are of concern, then the next step would be to determine the source of contamination. This is done by first identifying the sites that are most contaminated and then to identify point or non-point sources. It is also important to determine if there is a geographical and temporal variation in contamination. With geographical variation, contamination at one site might be washed to other sites, for example down a river. With temporal variation, it is important to determine if contamination occurs when pesticides are applied during irrigation times, or at first rainfall.

HOW TO MANAGE PESTICIDE WATER POLLUTION

Under the USEPA framework, interventions aimed at remediation of contaminated groundwater are triggered by the number of sampling sites with detections and the frequency of these detections.[13] Management measures proposed by EPA are listed in Table 2.

Table 2: Pesticide Management Measures proposed by EPA

Moratorium areas
Wellhead protection areas
Buffer zones: location, depth and construction only for new wells
Change in practice of applications (time, rate and method)
Restrict area or form of application, or no application permitted at all
Advance notice of application
Best Management Practices
Integrated pest management
Training and certification

To protect groundwater, geographic restrictions on pesticide use, and buffer zones near water bodies where pesticide use is prohibited may be useful to protect surface water.[14] Restrictions have played an important role in response to evidence of groundwater contamination throughout Organisation for Economic Co-operation and Development (OECD) countries. These restrictions ranged from regulations reducing use rates, or setting conditions for application, through to outright bans. **Such restrictions have sought to reverse the trend of contamination by atrazine found in many studies.**
Other measures such as buffering have played an important role. Preventive measures such as tightening of restrictions on filling and cleaning of spray equipment have also been successfully pursued.

One of the many options at a policy level for addressing pesticide pollution is to levy a tax that aims to reduce usage and therefore the potential for exposure. Huang and Uri[15] have modelled the level at which such a tax might be set in order to reach the optimal trade-off between adequate agricultural production and limiting adverse environmental impacts on water sources. Although such measures are relatively blunt tools for controlling water pollution, they should still be considered in the scope of regulatory and policy interventions to be considered for protecting South Africa's water quality. Taxation as a tool for discouraging unnecessary use has not been widely applied in OECD countries, where economic instruments such as green labelling and environmentally-linked subsidies have been widely enforced.

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ADDENDUM A:

SUMMARY OF INTERNATIONAL STANDARDS FOR PESTICIDES IN DRINKING WATER (mg/l)

Summary of International Standards for Pesticides in drinking water (mg/ℓ)

Type of Standard:	*USEPA*							*Australian*	*WHO*
	MCL standard	*Child Advisories*			*Adult Advisories*			*Advisory at 10-4 Cancer Risk*		
		1 day	*10 day*	*Longer term*	*Longer term*	*DWEL*	*Lifetime*			
Pesticide		***Child***	***Child***	***Child***	***Adult***	***Adult***	***Adult***	***Adult***		
Acifluorfen		2	2	0.1	0.4	0.4		0.1		
Alachlor	0.002	0.1	0.1			0.4		0.04	0.002	0.02
Aldicarb	0.007					0.035	0.007		0.001	0.01
Aldicarb sulfone	0.007					0.035	0.007			
Aldicarb sulfoxide	0					0.035	0.007			
Aldrin			0.0003	0.0003	0.0003	0.001		0.0002	0.0003	0.0003
Ametryn		9	9	0.9	3	0.3	0.06		0.05	
Ammonium Sulfamate		20	20	20	80	8	2			
Atrazine	0.03	0.1	0.1	0.05	0.2	0.2	0.003		0.02	0.002
Bentazon		0.3	0.3	0.3	1	1	0.2		0.03	0.03
Bromacil		5	5	3	9	5	0.09		0.3	
Butylate		2	2	1	4	2	0.35			
Carbaryl		1	1	1	1	4	0.7		0.03	
Carbofuran	0.04	0.05	0.05	0.05	0.2	0.2	0.04		0.01	0.005
Carbon tetrachloride	0.005	4	0.2	0.07	0.3	0.03		0.03	0.003	
Chloramben		3	3	0.2	0.5	0.5	0.1			
Chlordane	0.002	0.06	0.06			0.002		0.003	0.001	0.0002
Chlorpyrifos		0.03	0.03	0.03	0.1	0.1	0.02		-	
2,4-D	0.07								0.03	0.03
DCPA		80	80	5	20				-	
Diazinon		0.02	0.02	0.005	0.02	0.003	0.0006		0.003	
Dieldrin		0.0005	0.0005	0.0005	0.002	0.02		0.0002	0.0003	
Diphenamid		0.3	0.3	0.3	1	1	0.2		0.3	
Diphenylamine		1	1	0.3	1	1	0.2		-	
Diquat	0.02					0.08	0.02		0.005	
Disulfoton		0.1	0.1	0.003	0.009	0.001	0.0003		0.003	
Endosulfan									0.03	
Endothall	0.1	0.8	0.8	0.2	0.2	0.7	0.1		0.1	

Type of Standard:	***USEPA***								***Australian***	***WHO***
	MCL standard	***Child Advisories***			***Adult Advisories***			***Advisory at 10-4 Cancer Risk***		
		1 day	***10 day***	***Longer term***	***Longer term***	***DWEL***	***Lifetime***			
Pesticide		***Child***	***Child***	***Child***	***Adult***	***Adult***	***Adult***	***Adult***		
Ethylene dibromide	0.00005	0	0	0				0.00004	0.001	
Fenamiphos		0.009	0.009	0.005	0.002	0.09	0.002		0.0003	
Fonofos		0.02	0.02	0.02	0.07	0.07	0.01		-	
Glyphosate	0.7	20	20	1	1	4	0.7	-	1.0	
Heptachlor	0.0004	0.01	0.01	0.005	0.005	0.02		0.0008	0.0003	0.00003
Heptachlor epoxide	0.0002	0.01	0.01	0.0001	0.0001	0.0004		0.0004	0.0003	0.00003
Hexachlorobenzene	0.001	0.05	0.05	0.05	0.2	0.03		0.002		0.001
Hexachlorocyclopentadiene	0.05					0.2				
Lindane	0.0002	1	1	0.03	0.1	0.01	0.0002		0.00005	0.002
Malathion		0.2	0.2	0.2	0.8	0.8	0.2			
MCPA			0.1	0.1	0.4	0.05	0.01			0.002
Methomyl		0.3	0.3	0.3	0.3	0.9	0.2		0.03	
Methoxychlor	0.04	0.05	0.05	0.05	0.2	0.2	0.04		0.3	0.02
Methyl parathion		0.3	0.3	0.03	0.1	0	0			
Metolachlor		2	2	2	5	3.5	0.07		0.3	0.01
Metribuzin		5	5	0.3	0.5	0.5	0.1		0.05	
Naphthalene		0.5	0.5	0.5	1	0.1	0.02			
Oxamyl	0.2	0.2	0.2	0.2	0.9	0.9	0.2		0.1	
Paraquat		0.1	0.1	0.05	0.2	0.2	0.03		0.03	
Pentachlorphenol	0.001	1	0.3	0.3	1	1		0.03	0.01	0.009
Picloram	0.5	20	20	0.7	2	2	0.5		0.3	
PCB's	0.0005							0.0005	0.0005	
Prometon		0.2	0.2	0.2	0.5	0.5	0.1			
Pronamid				0.8	3					
Propachlor		0.5	0.5	0.1	0.5	0.5	0.09			
Propazine		1	1	0.5	2	0.7	0.01		0.05	

Type of Standard:	***USEPA***								***Australian***	***WHO***
	MCL standard	***Child Advisories***			***Adult Advisories***			***Advisory at 10-4 Cancer Risk***		
		1 day	***10 day***	***Longer term***	***Longer term***	***DWEL***	***Lifetime***			
Pesticide		***Child***	***Child***	***Child***	***Adult***	***Adult***	***Adult***	***Adult***		
Simazine	0.004	0.004	0.07	0.07	0.07	0.2	0.004		0.02	0.002
Tebuthiuron		3	3	0.7	2	2	0.5			
Terbacil		0.3	0.3	0.3	0.9	0.4	0.09		0.03	
Terbufos		0.005	0.005	0.001	0.005	0.05	0.0009		0.0005	
Toxaphene	0.003							0.003	0.003	
Trifluralin		0.08	0.08	0.08	0.3	0.3	0.005	0.5	0.05	0.02

Sources:

National Health and Medical Research Council, and Agriculture and Resource Management Council of Australia and New Zealand, 1996;
USEPA 1986; WHO, 1993b

MCL : Maximum Contaminant Level
DWEL : Drinking Water Equivalent Level

ADDENDUM B:

PESTICIDES LISTED AS POTENTIAL ENDOCRINE DISRUPTORS

WORLD WILD LIFE FUND CANADA lists the following as endocrine disruptors
(Source: http://www.wwfcanada.org/hormone-disruptors/science/frameset.html)

2,4,5-T	DBCP	h-epoxide	nitrofen
2,4-D	DDT	kelthane	oxychlordane
alachlor	DDT metabolites	kepone	permethrin
aldicarb	dicofol	malahion	synthetic pyrethroids
amitrole	dieldrin	mancozeb	toxaphene
atrazine	endosulphan	maneb	transnonaachlor
benomyl	esfenvalerate	methoxymyl	tributyltin oxide
beta – HCH	ethylparathion	methoxychlor	trifluralin
carbaryl	fenvalerate	metiram	vinclozolin
chlordane	lindane	metribuzin	zineb
cypermethrin	heptachlor	mirex	ziram

The **German list of endocrine disruptors** (Bruhn et al, 1998)

Endocrinely Active	**Potentially Endocrinely Active**
Atrazine	2,4,5 Trichlorophenoxyacetic acid
2,4 dichlorophenoxyacetic acid	Ziram
amitrole	Metiram
metribuzin	Nonachlor (*cis*- and *trans*-)
Nitrofen	Aldrin
Trifluralin	Dicofol
Thiram	Pyrethroids
Maneb	2,4 Dicholorphenol
Zineb	
Macozeb	
HCB	
PCP	
Fenarimol	
Carbaryl	
Chlordane	
β-HCH	
γ-HCH	
Parathion	

US **Physicians for Social Responsibility** - List of known or suspected reproductive and developmental toxins, used in Massachusetts (Schettler et al, 1996)

parathion	benomyl	dicofol
malathione	maneb	cypermethrin
diazanon	nabam	fenvelerate
chlorpyrifos	zineb	ethylene bromide
tetrachlorvinphos	thiram	ethylene oxide
acephate	vinclozin	dicamba
dimethioate	paraquat	methoxychlor
carbaryl	atazine	2,4 D
lindane	cyanazine	
endosulphan	dieldrin	