THE DEVELOPMENT OF AUTOMATED METHODS FOR THE
DETERMINATION OF TRACE CONCENTRATIONS OF
CARBAMATE PESTICIDES IN WATER USING SOLID SORBENT
PRE-CONCENTRATION METHODS AND HIGH PERFORMANCE
LIQUID CHROMATOGRAPHY

By
Christopher H. Marvin

A Thesis

Presented to the Department of Chemistry in Partial
Fulfillment of the Requirements for the Degree of
Master of Science

May, 1990
Brock University
St. Catharines, Ontario

© C.H. Marvin, 1990
ABSTRACT

Several automated reversed-phase HPLC methods have been developed to determine trace concentrations of carbamate pesticides (which are of concern in Ontario environmental samples) in water by utilizing two solid sorbent extraction techniques. One of the methods is known as on-line pre-concentration. This technique involves passing 100 milliliters of sample water through a 3 cm pre-column, packed with 5 micron ODS sorbent, at flow rates varying from 5-10 mL/min. By the use of a valve apparatus, the HPLC system is then switched to a gradient mobile phase program consisting of acetonitrile and water. The analytes, Propoxur, Carbofuran, Carbaryl, Propham, Captan, Chlorpropham, Barban, and Butylate, which are pre-concentrated on the pre-column, are eluted and separated on a 25 cm C-8 analytical column and determined by UV absorption at 220 nm. The total analytical time is 60 minutes, and the pre-column can be used repeatedly for the analysis of as many as thirty samples. The method is highly sensitive as 100 percent of the analytes present in the sample can be injected into the HPLC. No breakthrough of any of the analytes was observed and the minimum detectable concentrations range from 10 to 480 ng/L. The developed method is totally automated for the analysis of one sample.

When the above mobile phase is modified with a buffer solution, Aminocarb, Benomyl, and its degradation product, MBC, can also be detected along with the above pesticides with baseline resolution for all of the analytes. The method can also be easily
modified to determine Benomyl and MBC both as solute and as particulate matter.

By using a commercially available solid phase extraction cartridge, in lieu of a pre-column, for the extraction and concentration of analytes, a completely automated method has been developed with the aid of the Waters Millilab Workstation. Sample water is loaded at 10 mL/min through a cartridge and the concentrated analytes are eluted from the sorbent with acetonitrile. The resulting eluate is blown-down under nitrogen, made up to volume with water, and injected into the HPLC. The total analytical time is 90 minutes. Fifty percent of the analytes present in the sample can be injected into the HPLC, and recoveries for the above eight pesticides ranged from 84 to 93 percent. The minimum detectable concentrations range from 20 to 960 ng/L. The developed method is totally automated for the analysis of up to thirty consecutive samples. The method has proven to be applicable to both purer water samples as well as untreated lake water samples.
ACKNOWLEDGEMENTS

I would like to take his opportunity to express my deepest gratitude to my supervisor, Dr. Mikio Chiba of Agriculture Canada, for his guidance throughout the course of this study.

I would also like to thank Prof. Ian D. Brindle for his guidance during my course of study at Brock University. The author also wishes to thank Dr. C. David Hall of the Ontario Ministry of the Environment.

I would also like to thank Donna Vukmanic, Tim Jones, John Vandenhoff, Dr. M.S. Gibson, Gail Neff, Dr. J. Miller, John Rustenburg, and the staff of the machine and electronics shops at Brock.
TABLE OF CONTENTS

Abstract
Acknowledgements
List of Figures
List of Tables

INTRODUCTION

A. SOLID PHASE EXTRACTION
B. ON-LINE PRE-CONCENTRATION
C. CARBAMATE PESTICIDES
D. BENOMYL
E. SAMPLE PREPARATION AUTOMATION
F. SCOPE OF THE STUDY

EXPERIMENTAL SECTION

A. ON-LINE PRE-CONCENTRATION
   -Solvents
   -Pesticides
   -Preparation of Stock Solutions
   -Water Samples
   -HPLC Apparatus
   -Operating Conditions
   -On-line Pre-concentration
B. SOLID PHASE EXTRACTION

- Solvents 39
- Pesticides 39
- Preparation of Standard Solutions 39
- Water Samples 39
- HPLC Apparatus 40
- SPE Cartridges and Analytical Column 40
- Operating Conditions 40
- Gradient Elution Program 40
- SPE Procedure 41

C. BENOMYL

- Solvents and preparation of mobile phase 42
- Pesticides 42
- Preparation of Stock Standard Solutions 42
- Water Samples 43
- HPLC Apparatus 43
- Operating Conditions 44
- On-line Pre-concentration 44
- Elution 44
- Preparation of Buffered Mobile Phase 45
RESULTS AND DISCUSSION

A. PRELIMINARY STUDIES

- Initial Experiments with Carbaryl. 55
- Preliminary On-line Pre-concentration experiments with Carbamate Pesticides 62
- Initial On-line Pre-concentration Experiments Using Municipal Tap Water. 62
- Summation of Preliminary Studies 69

B. ON-LINE PRE-CONCENTRATION

- Unidirectional and Backflush Elution 70
- Statistical Calculations of Column Performance 74
- C-8 and C-18 Stationary Phases 78
- Minimum Detectable Concentrations 81
- Size of Sorbent in Pre-column 83
- Sample Loading Rate 85
- Pre-column Longevity 89
- Sample Matrix Study 91
- Instrument Calibration 91

C. SOLID PHASE EXTRACTION

- Sample Loading Rate 97
- Cartridge Packing Size 100
- Choice of Eluting Solvent 100
- Weight of Solid Sorbent Contained in the Cartridge 103
- Study of Residual Water on the Cartridge 107
- Sample Matrix Study 123

**D. BENOMYL** 123

- Inclusion of Buffered Mobile Phase 127
- Study of Experimental Conditions 127
- Analysis of Benomyl as both Solute and in the Solid State. 130
- Optimum Conditions for the analysis of Benomyl and MBC only. 135

**SUMMARY AND CONCLUSIONS** 137

**A. ON-LINE PRE-CONCENTRATION** 137

**B. SOLID PHASE EXTRACTION** 139

**C. ANALYSIS OF BENOMYL** 139

**Appendix 1** 141

Listing of the Carbamate Pesticides, alternative names, history, solubilities, toxicities, and methods of analysis.

**Appendix 2** 146

The development of an SPE method for the determination of chlorophenols and phenoxy-acids in water.

**Appendix 3** 155

Standard calibration data and curves for the eight pesticides selected for the on-line pre-concentration and SPE studies.
Appendix 4 176

The pesticides making up MOE combined standard 1 (MOE 1).

Appendix 5 177

The pesticides making up MOE combined standard 4 (MOE 4).

Appendix 6 178

The pesticides making up MOE combined standard 7 (MOE 7).

REFERENCES 179
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fig. 1.</strong></td>
<td>Proposed mechanisms for the hydrolysis of N-methylcarbamates (36,37).</td>
</tr>
<tr>
<td><strong>Fig. 2.</strong></td>
<td>Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1.</td>
</tr>
<tr>
<td><strong>Fig. 3.</strong></td>
<td>Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1.</td>
</tr>
<tr>
<td><strong>Fig. 4.</strong></td>
<td>Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1. The optimum gradient for a C-18 analytical column is marked with an asterisk.</td>
</tr>
<tr>
<td><strong>Fig. 5.</strong></td>
<td>Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1.</td>
</tr>
<tr>
<td><strong>Fig. 6.</strong></td>
<td>Chromatogram showing the best separation of the analytes in MOE 1 obtained from an 80 microliter injection of MOE 1 diluted approximately 10:1 in water. The chromatogram was produced using the developed C-18 gradient program and plotted at 300 millivolts.</td>
</tr>
<tr>
<td><strong>Fig. 7.</strong></td>
<td>Chromatogram showing the results obtained from a 20 microliter injection of approximately 10 ppm of Diallate and 10 ppm Butylate in water. The analytes were separated on a C-18 analytical column isocratically (70% acetonitrile/30% water) at a flow rate of 1 mL/min. The chromatogram was plotted at 50 millivolts.</td>
</tr>
<tr>
<td><strong>Fig. 8.</strong></td>
<td>Ultra-violet scan of MOE 1 diluted approximately 200:1 in water.</td>
</tr>
<tr>
<td><strong>Fig. 9.</strong></td>
<td>Chromatogram resulting from the analysis of an 80 microliter aliquot of an acetonitrile solution in which a rubber stopper was immersed for 12 hours. The chromatogram was produced using a C-18</td>
</tr>
</tbody>
</table>
analytical column with the developed C-18 gradient program. The chromatogram was plotted at 300 millivolts.

**Fig. 10.** Chromatogram resulting from the analysis of MOE 1 diluted approximately 10:1 in water. The analysis was performed on a C-8 analytical column using the developed method.

**Fig. 11.** Chromatogram resulting from an 80 microliter injection of MOE 1 diluted approximately 10:1 in water. The analysis was performed on a C-8 analytical column using those conditions found optimum for the C-18 analytical column (gradient C-18). The chromatogram was plotted at 250 millivolts.

**Fig. 12.** Chromatogram resulting from the on-line pre-concentration of 100 mL of sample prepared by the dilution of MOE 4 1000:1 in distilled water. The sample loading rate was 2.5 mL/min. Separation was performed on a C-8 analytical column using the developed method. The chromatogram was plotted at 200 millivolts.

**Fig. 13.** Chromatogram resulting from the pre-concentration of 100 mL of municipal tap water using the described method. The chromatogram was plotted at 200 millivolts.

**Fig. 14.** Chromatogram resulting from the pre-concentration of a 100 mL sample prepared by the 1000:1 dilution of MOE 7 in water. The sample was loaded onto a 3 cm 5 micron C-18 pre-column at 2.5 mL/min and analysed by the described method with the exception that backflush elution was employed.

**Fig. 15.** Schematic of the valve-switching system. V, P, and F denote valves, pumps, and filters respectively. During the sample loading step, P1 dispenses sample. During the elution steps, P1 dispenses water and P2 acetonitrile as part of the mobile phase.
Fig. 16. Chromatograms showing the effect of backflush elution (16A) and unidirectional elution (16B) for a 5 micron C-18 pre-column. Each chromatogram is plotted at 90 millivolts. The samples were prepared and analysed by the method described in the experimental section.

Fig. 17. Chromatogram resulting from a 150 microliter injection of the spiking standard diluted to 30% acetonitrile in water and analysed by the developed method.

Fig. 18. Chromatogram resulting from the pre-concentration of 100 mL of sample and subsequent elution of the analytes directly to the UV detector without passing through an analytical column. The chromatogram was produced using the developed method and plotted at 150 millivolts.

Fig. 19. Chromatograms showing the effect of backflush (19A) and unidirectional elution (19B) for a 5 micron C-8 pre-column. Each chromatogram is plotted at 90 millivolts. The samples were analysed by the developed method.

Fig. 20. Chromatograms corresponding to a blank (20B), sample (20A), and background subtraction (20C) from a distilled water sample pre-concentrated on a 10 micron Ultrasil ODS pre-column. The concentrations of the pesticides are one tenth those listed in Table 10. Each chromatogram is plotted at 90 millivolts. The samples were analysed by the developed method.

Fig. 21. Chromatograms showing the effect of sorbent particle diameter upon the retention of analytes. Chromatograms correspond to 40 micron (21A), 10 micron (21B), and 5 micron (21C) packings. Each chromatogram is plotted at 90 millivolts. The samples were
analysed by the developed method.

Fig. 22. Chromatograms showing the effect of sample loading rate upon retention of analytes for a 5 micron C-18 pre-column. Chromatograms correspond to 3 mL/min (22A), 4 mL/min (22B), 5 mL/min (22C), and 6 mL/min (22D). Chromatograms are plotted at 90 millivolts. The samples were analysed by the developed method.

Fig. 23. Chromatograms showing the effect of sample loading rate upon retention of analytes for a 10 micron C-18 pre-column. Chromatograms correspond to 4 mL/min (23A), 5 mL/min (23B), 6 mL/min (23C), and 7 mL/min (23D). Chromatograms are plotted at 90 millivolts. The samples were analysed by the developed method.

Fig. 24 Chromatogram resulting from an analysis in which the sample loading rate was 10 mL/min. The concentrations of the pesticides were the same as those listed in Table 10 except for Propoxur, which was present in the sample at 3.5 ppb. The sample was analysed by the developed method.

Fig. 25. Chromatogram showing the effect of prolonged use of a 5 micron C-18 cartridge. The sample was analysed by the developed method. The chromatogram was plotted at 90 millivolts.

Fig. 26. Chromatograms of distilled (26A), one of the best HPLC grade (26B), and reverse osmosis (26C) waters. Each chromatogram is plotted at 15 millivolts. The samples were analysed by the developed method.

Fig. 27. Chromatograms of two commercial bottled spring waters. Chromatogram (27A) is a Canadian product and
chromatogram (27B) corresponds to a European product.
Each chromatogram is plotted at 40 millivolts. The samples were
analysed by the developed method.

**Fig. 28.** Chromatograms comparing two municipal tap waters. Each
chromatogram is plotted at 40 millivolts. The samples were
analysed by the described method.

**Fig. 29.** Chromatograms comparing laboratory distilled (29A), and
deionized (29B) waters. Chromatograms are plotted at 40 millivolts.
The samples were analysed by the developed method.

**Fig. 30.** Chromatograms showing a comparison of two other commercial
HPLC grade waters. Chromatograms are plotted at 40 millivolts.
The samples were analysed by the developed method.

**Fig. 31.** Chromatogram resulting from the analysis of a sample containing
ten times the concentration of those listed in Table 10 (with the
exception of Propoxur which is present at 3.3 ppb). The sample
was analysed by the developed method.

**Fig. 32.** Chromatogram resulting from the on-line pre-concentration
analysis of the sample effluent from the Sep-Pak Plus cartridge
during a sample loading step where the flow rate through the SPE
cartridge was 20 mL/min. The sample was analysed by the
developed on-line pre-concentration method.

**Fig. 33.** A comparison of the retention of analytes by (33A) 40 micron
C-18 (Sep-Pak Custom cartridge) and (33B) 90 micron C-18
(Sep-Pak Plus cartridge). Chromatograms were produced by
injection of 0.175 mL of eluate without a blow-down step.
Chromatograms are plotted at 25 millivolts. The sample was
analysed by the developed method.
Fig. 34. Chromatogram resulting from the analysis of a municipal tap water using the developed SPE methodology and the Sep-Pak Plus cartridge.

Fig. 35. Chromatogram resulting from the analysis of a municipal tap water using the described SPE methodology and the Sep-Pak Light cartridge.

Fig. 36. Chromatogram resulting from the injection of a 175 microliter aliquot of the eluate from the Sep-Pak Light cartridge before the evaporation step under nitrogen. Otherwise, the developed method is used.

Fig. 37. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 20% acetonitrile in water using the developed method.

Fig. 38. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 30% acetonitrile in water using the developed method.

Fig. 39. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 40% acetonitrile in water using the developed method.

Fig. 40. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 50% acetonitrile in water using the developed method.

Fig. 41. Chromatograms showing the relative amounts of the analytes removed from the Sep-Pak Light cartridge by successive 250 microliter acetonitrile eluant volumes. Chromatogram (41A) corresponds to the first 250 microliters and chromatogram (41C) to the last 250 microliters. The samples were analysed by the
developed method.

**Fig. 42.** Chromatogram resulting from the analysis of a sample where the eluant was blown-down under nitrogen to a volume of 100 microliters. The sample was analysed by the developed method.

**Fig. 43.** Chromatogram of a combined standard solution sample prepared in the best commercially available HPLC grade water. The analysis was done by the developed method.

**Fig. 44.** Chromatograms resulting from three successive injections of the combined standard sample solution by following the developed method. Chromatograms were plotted at 50 millivolts and gradient correction was made.

**Fig. 45.** Chromatogram resulting from the analysis of an untreated lake water. The sample was analysed by the developed method. This can be compared with Fig. 36 showing the analysis of a municipal tap water.

**Fig. 46.** Chromatogram showing the eleven pesticides used in the Benomyl, MBC study. The sample was analysed by the developed method. The concentrations of the pesticides are the same as those listed in Table 14.

**Fig. 47.** Chromatogram resulting from the analysis of a sample containing 1.3 ppb Benomyl. The sample was analysed by the developed method. MBC is present in the sample as the degradation product of Benomyl.

**Fig. 48.** Chromatogram resulting from the analysis of a sample by the developed method with the exception that a C-8 analytical column was used.

**Fig. 49.** Chromatogram resulting from a straight injection onto the analytical column of a sample containing only Benomyl and MBC. The chromatogram was plotted at 75 millivolts. The sample was
analysed by the developed method.

**Fig. 50.** Chromatogram resulting from the analysis of a 0.136 ppm Benomyl suspension. A 10 mL sample was analysed by the developed method except that backflush elution was employed.

**Fig. 51.** Chromatogram resulting from the analysis, by the developed method, of the same 0.136 ppm Benomyl suspension described in Fig. 51, with the exception that the sample was analysed 45 minutes later.

**Fig. 52.** Chromatogram resulting from the analysis of a sample containing Benomyl and MBC only. The gradient used is that developed for the C-18 analytical column and is described in the on-line pre-concentration experimental section. The use of this gradient results in a faster increase in the percentage of acetonitrile in the mobile phase and substantially sharper MBC and Benomyl peak profiles.
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Rate constants for the hydrolysis of substituted N-methylcarbamates and phenyl N-substituted carbamates.</td>
<td>23</td>
</tr>
<tr>
<td>Table 2</td>
<td>Peak areas obtained from incremental injections of a Carbaryl standard using the developed method.</td>
<td>59</td>
</tr>
<tr>
<td>Table 3</td>
<td>Peak areas of Carbaryl from three consecutive on-line pre-concentration samples using the developed method.</td>
<td>61</td>
</tr>
<tr>
<td>Table 4</td>
<td>Peak area data from three replicate samples containing the pesticides from MOE 4 using the developed method.</td>
<td>63</td>
</tr>
<tr>
<td>Table 5</td>
<td>Peak area data from 5 replicate samples containing the pesticides from MOE 7 using the developed method.</td>
<td>68</td>
</tr>
<tr>
<td>Table 6</td>
<td>The retention times, capacity factors, and theoretical plate numbers for each of the compounds in MOE 10. The calculations were made using the results of a straight injection of MOE 10 using the developed method.</td>
<td>75</td>
</tr>
<tr>
<td>Table 7</td>
<td>The retention times, capacity factors, and theoretical plate numbers for each of the compounds in MOE 10. The calculations were made using the results of an on-line pre-concentration sample run prepared using MOE 10 and the developed method.</td>
<td>76</td>
</tr>
<tr>
<td>Table 8</td>
<td>The retention times, average peak area counts, MOE 10 sample concentrations, and minimum detectable concentrations for replicate on-line pre-concentration sample runs using the developed method.</td>
<td>80</td>
</tr>
<tr>
<td>Table 9</td>
<td>Tabulation of average peak area counts over three orders of concentration for on-line pre-concentration samples using the developed method.</td>
<td>98</td>
</tr>
<tr>
<td>Table 10</td>
<td>Average recovery of Butylate in relation to the final volume of eluate after blow-down.</td>
<td>119</td>
</tr>
</tbody>
</table>
The samples were prepared using the developed method.

**Table 11.** The retention times, recoveries, MOE 10 sample concentrations, and minimum detectable concentrations for replicate SPE sample runs using the developed method.

**Table 12.** The retention times, sample concentrations, and minimum detectable concentrations, for the pesticides selected for the Benomyl study. The samples were analysed by the developed method.
INTRODUCTION

In recent years, much attention has been focussed on the development of improved sample preparation techniques as alternatives to conventional methods such as liquid-liquid extraction. Methods used by the Ontario Ministry of the Environment for the extraction of organic pollutants from water sample matrices employ a liquid-liquid extraction step (1,2). Statistically, the efficiency of a liquid-liquid extraction is dependent upon the partition coefficient (equation 1) and the number of successive extractions performed (3). As seen from equations 1 and 2, a greater number of extractions with smaller volumes of solvent is a more efficient method of extraction than a single extraction with a greater volume of solvent.

\[ K \left( \frac{[A]_{\text{org}}}{[A]_{\text{aq}}} \right) = \frac{V_{\text{aq}}}{V_{\text{org}} K + V_{\text{aq}}} \]  

Where (A) denotes the concentration of the analyte

\[ [(A)_{\text{aq}}]_n = \left( \frac{V_{\text{aq}}}{V_{\text{org}} K + V_{\text{aq}}} \right)^n [(A)_{\text{aq}}]_0 \]  

where (A) is the concentration of analyte, [(A)\text{aq}]_0 denotes the initial concentration of the analyte in the aqueous phase, and n is the number of extractions.

Liquid-liquid extraction can be performed manually by the use of a separatory funnel but generally requires high partition coefficient values for the analytes. The technique can be automated
through the use of a continuous (or exhaustive) extraction apparatus or a countercurrent extraction (4, 5) apparatus. Both techniques require periods of at least several hours for complete extraction but can separate components even when the values of the partition coefficients are unfavourable (3). Countercurrent extractors can separate components whose partition coefficients differ by less than 0.1 (6). Unfortunately, the countercurrent extractors commercially available are very large in size.

Coupled with the time factor is the fact that a large volume of organic solvent is required to achieve a satisfactory liquid-liquid extraction. This solvent may require further clean-up (1), followed by evaporation (and possibly reconstitution) to a volume suitable for analysis (1). Evaporation of large volumes of solvent can result in the loss of volatile components. There is also growing concern about the safety and expense of utilizing large volumes of organic solvents such as dichloromethane, that are employed in liquid-liquid extractions. These factors, combined with the fact that it is difficult to extract polar substances from water, make the determination of trace concentrations at or below the parts per million (ppm = 10^{-6} g/mL) level, difficult, and time and labour intensive.

Since the early 1970's, the accurate determination of organic pollutants in water has become the chief objective of many analytical chemists. Pollutants in untreated lake and river waters are often present at the parts per billion (ppb = 10^{-9} g/mL) level and in drinking water at the parts per trillion (ppt = 10^{-12} g/mL) level. This results in the need for sample preparation techniques that can
sufficiently concentrate analytes to the point that the aforementioned concentrations can be determined.

One technique, investigated as an alternative to liquid-liquid extraction, involves adsorption onto the surface of a solid sorbent. Investigation of this technique as early as 1951 (7), led to current solid sorbent technology. This technology has received much attention in recent years. The use of a solid sorbent should, in theory, result in a more efficient extraction of analytes. The amount of organic solvent needed for the procedure is greatly reduced as is the period of time required. Two very similar techniques employing a solid sorbent for the pre-concentration of organic analytes are solid phase extraction (SPE) and on-line pre-concentration (or trace enrichment).

SPE involves passing a required volume (often several hundred mL) of sample through a cartridge containing a selected amount of solid sorbent. During this procedure, the analytes are extracted and retained by the sorbent. In recent years, most laboratories use commercially available pre-packed cartridges which contain fixed amounts of desired types of sorbent for this purpose. The analytes thus adsorbed are eluted from the cartridge by a small volume (usually several hundred microliters to several mL) of organic solvent. The resultant solution, which is substantially concentrated and often purified, can be evaporated (if desired) and reconstituted in an appropriate solvent for determination by gas chromatography (GC) or high performance liquid chromatography (HPLC) analysis.

Modern HPLC is one of the most widely used methods for the separation of complex mixtures of organic analytes in varying
sample matrices. A closed, reusable, stainless steel column containing a selected amount of a desired stationary phase is subjected to a flow of liquid mobile phase of varying solvent strength and composition. The mobile phase is introduced to the column by mechanical pumps capable of delivering precise volumes in a given period of time at very high pressures (typically 500-5000 psi). Separation of the analytes results from interactions between sample molecules and the stationary and mobile phases (8). Effluent from the column is then directed to a detector, usually an ultraviolet (UV) or fluorescence detector.

When SPE is combined with HPLC, by placing the solid sorbent 'on-line', a technique known as on-line pre-concentration results. Usually a short pre-column (3-5 cm), which is located in front of the analytical column and termed the pre-column, is used as a solid phase sorbent. After the pre-concentration of analytes on the pre-column, an appropriate mobile phase is introduced to the system. The analytes are eluted from the pre-column and led directly onto the analytical column. In order to perform the above total procedure of on-line pre-concentration and elution in conjunction with an ordinary HPLC system, a simple valve switch is all that is required.

A. SOLID PHASE EXTRACTION

Adsorption onto a solid substrate as a method of pre-concentration had been documented as early as the 1950's. In 1951, Middleton and Walton (7) utilized granular activated carbon for the concentration of organics in drinking water. This was accomplished
by passing 5,000 to 75,000 gallons of water through carbon filters at the rate of 0.1 to 0.6 gallons per minute. The filter itself was a three foot length of iron piping of 4 inch internal diameter containing 1200-1500 g of 4-10 mesh granular carbon. The filters were then extracted with ether in a soxhlet extractor followed by a series of liquid-liquid extractions to separate the extract into phenolic, acidic, and amphoteric compounds.

Despite the obvious shortcomings of the technique, including the use of a coarse granular carbon mesh and the many liquid-liquid extraction steps, the detection limits ranged from 10-70 ppb. The method was also applied to the determination of chlorinated insecticides in surface waters in the late 1950's (9). Sample and filter sizes were reduced to a laboratory scale (10-30 L samples, 12 g filters). The method demonstrated 91-100 % adsorbtion efficiency and 75-86 % recoveries for insecticides such as DDT, Aldrin, and Endrin. Detection limits of the order of 10 ppb were obtained in conjunction with LC and infrared (IR) spectrophotometry.

The development of this technique led to the standardised methods of carbon/chloroform extraction (CCE) and carbon/alcohol extraction (CAE). These techniques proved advantageous in that carbon has a high adsorbtion capacity (permitting the use of small amounts of sorbent for extractions) and a high thermal stability (up to 700 degrees centigrade). The most significant limitations of the method are losses of analyte by incomplete adsorbtion on the carbon substrate, incomplete elution by the organic solvent (usually alcohol or ether), and evaporative losses. It was also found that compounds desorbed from the carbon sorbent are not always identical to those
that are extracted from the water sample resulting in interferences and erroneous recoveries.

Junk and Richard (10) were among the many developers of porous organic polymer resins as solid sorbents. The most popular of these were the Rohm and Haas Amberlite XAD series (XAD-1 to XAD-8) of resins. These resins are low polarity styrene-divinylbenzene copolymers with high sorptive capacities. Recovery efficiencies from water were investigated with pesticides, amino acids, aliphatic acids, and other miscellaneous compounds.

Junk and Richard applied the method to the analysis of contaminants in natural and processed water samples (10). The analysis procedure involved a glass column (0.6 cm internal diameter by 10 cm long) packed with 20-60 mesh XAD-2 resin. Sample flow rates of 30-50 mL/min were employed followed by elution with diethyl ether, drying with anhydrous sodium sulphate, and concentration of the eluate by evaporation of the solvent. Subsequent analysis was performed by GC-mass spectrometry. For a set of 110 samples, the average recovery of organics was 78% with a standard deviation of 6.3%.

These results indicate that the procedure is sensitive, reproducible, and accurate. There are several basic factors that make the use of organic porous polymers advantageous for the extraction of trace pollutants from water:

1. If the proper polymer substrate is chosen, the partition coefficient(s) for a chosen compound(s) tends towards infinity for a polymer-water system. This introduces an element of specificity to the technique as illustrated by Chriswell et al.(11).
2. Adsorption of water onto the polymer substrate is minimal.
3. Conditioning of the polymer with water allows intimate contact between the compound of interest and the polymer surface.
4. The polymer itself is chemically inert.

Although the use of polymer resins represented a marked improvement over the use of charcoal, considerable developmental work was still necessary to develop the optimum procedure. Junk and Richard found that recoveries of compounds were dependent upon variations in apparatus, technique, and experimental conditions (10). A separate study involving sterols in waste water found that a finer resin size (from 60 to 100 mesh) increased the recovery but that an increase in sample flow rate (from 3 to 7 mL/min) resulted in a decrease in recovery. It was also found that the XAD-8 resin favours aliphatic compounds over aromatic or cyclic compounds and that there is a preference for functional groups in the order methyl > carboxylic acid > aldehyde > alcohol > amine. Choice of sorbent and elution solvent systems continue to be the most important factors in modern SPE work.

In 1975, May (12) coupled the techniques of GC-MS and LC for the analysis of hydrocarbons in marine sediments and seawater. The scheme involves initial extraction of volatile components by dynamic headspace sampling (or vapour stripping), trapping of these compounds on a Tenax-GC packed pre-column, and subsequent analysis by GC or GC-MS. The non-volatile components remaining in the water sample are pumped through an LC pre-column packed with Waters Associates Bondapack C-18 and then analyzed by analytical LC. Bondapack C-18 is a superficially porous solid support (37-50
microns) with a covalently bonded stationary phase 18 carbon units in length. This is one of the first examples of the basic concept of SPE, the choice of a stationary phase bonded to a solid support to retain specific types of compounds. Elution of the analyte from the coupled column set-up was begun with 30:70 methanol/water at 3 mL/min and increased to 100% methanol over 40 minutes. Detection was by UV (245 nm) photometry. The Tenax-GC pre-column containing the volatile compounds was heated according to a temperature program with helium as a carrier gas.

May concluded that this technique offered many advantages. The time involved in the work-up of samples was reduced from days to hours and compounds with a wide range of molecular weights could be determined. The trapping of non-volatile components on the LC pre-column did not involve solvent extractions or solvents other than water. The use of the reversed phase pre-column allowed the extraction of analytes from large volumes of sample and the use of the more expensive analytical column only for the analysis of the compounds of interest. The eluate from the pre-column was relatively free of polar compound interferences and the nondestructive nature of the separation allowed for collection of the isolate for spectrometric analysis.

The recoveries of compounds ranged from 78 to 92% for the less volatile compounds (pyrene and phenanthrene respectively at 2 and 1.5 ppm). Conversely, the recovery of increasingly volatile compounds was poor. This was compensated for in part by the increased efficiency of the GC analysis as the volatility of the compounds increased. The authors were justifiably optimistic about
the potential of the method despite the relatively low recovery for phenanthrene and the high detection limits (2 ppm). The procedure did not involve any conditioning or washing of the pre-column. May also concluded that fewer sample transfer and concentration steps during collection and handling would be necessary criteria for determinations at ppb levels (12).

Other workers expanded upon this technique. Ogan et al.(13) utilized a pre-column for extraction which was packed with 40 micron Perkin-Elmer ODS-SIL-X-2 C-18 sorbent for the analysis of polycyclic aromatic hydrocarbons (PAH's) in aqueous samples. Unlike May (12), they were able to reach detection limits of the order of 0.2 ppb. Great care was taken in the sampling procedure to avoid adsorption onto container walls, including the use of teflon bottles. Interestingly, the samples were made up to 20% in methanol by volume (other aliphatic alcohols were also tried) in an attempt to decrease adsorption onto glass and metal surfaces. The mobile phase was 38% acetonitrile/15% methanol/47% water and the temperature was maintained at 65°C. The analytical column was 10 micron Perkin-Elmer C-18 and detection was by fluorimetry at 365 nm. It was not fully understood by the authors at the time of publication why the methanol dilution resulted in significantly greater recoveries. It was indicated that the methanol increased the solubility of the PAH's in aqueous solution, and the fact that the methanol was allowing greater interaction of the analytes with the stationary phase was suggested.

As mentioned previously, the detection limits achieved were as low as 0.2 ppb for a 400 mL sample. Recoveries ranged from 94
to 101%. It was also concluded that LC is the best technique for the determination of this type of compound due to the non-volatility of PAH's, the compatibility of the reversed phase with the water matrix, and the sensitivity of the fluorimetry detection employed. It should be noted that it was necessary to expose the system to the sample overnight to fully saturate all possible adsorption sites to obtain maximum sensitivity. For a mixture of five selected PAH's, only benzanthrone showed breakthrough at the 400 mL sample volume. All five compounds showed linear response over a concentration range of 1 to 140 ng/L.

The investigation of the trace enrichment of five model compounds in seawater by Saner et al. (14) is one of the first papers to report the use of factory packed SPE cartridges. Trace enrichment with hand-packed 30 micron ODS columns was compared with trace enrichment with Waters Sep-Pak cartridges packed with approximately 0.35 g of 70 micron Bondapack C-18 material. The Sep-Pak cartridges were rinsed prior to use with methanol, followed by water, acetonitrile, water, and then subjected to a final methanol rinse. Samples were applied at a flow rate of 100 mL/min. Elution was performed with 2 mL of methanol. The eluate was separated on an ODS analytical column with methanol at a flow rate of 1 mL/min as the mobile phase.

The review in the above paper (14) is quite extensive and several pertinent conclusions were reached:

1. The Sep-Pak C-18 cartridges are portable, simple to use, and are conveniently disposable.
2. The efficiency of extraction of five compounds (acetone, benzene, m-cresol, acetophenone, and toluene; chosen as their solubilities in water range from infinity to .05% by weight) was roughly proportional to their capacity factor (or K, the equilibrium ratio of the amount of analyte in the stationary phase to the amount in the mobile phase) and inversely proportional to their solubilities in water.

3. An increase in sample volume resulted in a decrease in extraction efficiency for all five compounds, regardless of whether concentration was varied or held constant.

4. Since the extraction efficiency is dependent upon sample volume, water solubility, and K value, these factors must be considered when quantitatively extracting from water.

5. The cartridges should prove useful when analysing for specific contaminants.

6. The extraction efficiency of acetophenone was constant over six orders of magnitude in concentration (0.01 to 1000 ppm).

   It was noted that attempting to predict extraction efficiency by use of K is suspect and that the actual compound of interest should be used to determine extraction efficiency. Also discussed was mutual zone solubility on the cartridge. This is defined as a loss of retained analyte arising from a "chemical/physical interaction between an adsorbed component and the water stream forced through the cartridge causing a desorption of adsorbate both during the extraction process itself and particularly during any follow-up rinses." Obviously, this effect would result in decreased efficiency
of the cartridge and would make the choice of sample volume all the more important.

Today, the use of SPE is expanding in conjunction with HPLC, the fastest growing of the analytical techniques. Bonded-phase silicas are utilized and are readily available from many manufacturers. These sorbents display great structural integrity, high surface area, and a wide selection of stationary phases that can be covalently bonded to the silica substrate (15-19). Theoretically, SPE should be both selective and efficient as the interactions are the same as those that take place on an analytical LC column. SPE can take advantage of any unique properties the compound of interest may possess to effect a separation from the sample matrix and any interferences. The properties of polarity or ion-exchange are most often used as the basis for separation. The analyst must also take into account the solubility of the compound of interest in polar and non-polar solvents, whether the isolate has the ability to form a reversible covalent bond with the sorbent, and to consider to what extent interfering compounds can compete for binding sites.

The suggested procedure for the isolation of a compound of interest is as follows:
1. The selection of an appropriate sorbent, i.e. a sorbent which retains the analyte quantitatively with as few interferences as possible. This involves identification of the major matrix components and the general matrix effects on the isolate. Sorbent selection is usually performed by the testing of standard solutions in a solvent as close as possible to the actual sample matrix. If recoveries are significantly lower than desired, then an alternate
sorbent must be chosen or the matrix conditions adjusted (e.g. adjustment of pH). The sorbent should also yield a clean extract of the blank matrix (i.e. a clean chromatogram in the area of interest where the analytes elute).

2. 'Activation' of the sorbent bed by a conditioning solvent.

3. The sample is passed through the cartridge by positive pressure, by centrifuge, or under vacuum.

4. The sorbent bed is washed with a suitable solvent to elute any remaining interfering compounds, but not the analyte.

5. Elution of the analyte by a suitable solvent. Solvents should be chosen that elute the analytes in the smallest possible volume. Solvents that do not elute the compound of interest are also identified as they can be used as wash solvents.

The technique offers many advantages over liquid-liquid extraction including increased selectivity and decreased sample and solvent volumes. The technique could be integrated into a totally automated system that many analytical chemists are attempting to develop. Samples could be taken at the field site and then transported to the laboratory for analysis with a minimal amount of wet chemistry or loss of sample.

The most pressing task at hand before SPE can be used with the utmost of confidence is the development of practical methodologies. As seen in the body of this section, there are a great number of factors that must be taken into account, from the physical interactions between solid phase, solvent, and analyte, to breakthrough volume. Some commercial manufacturers of SPE cartridges such as Supelco, include in the accompanying literature,
methods development flow charts to aid in choosing sorbents and solvents. These can seldom be used if one has to analyze a unique sample matrix. Once a suitable methodology has been developed, and the many experimental parameters optimized and understood, analysis using SPE should be more straightforward and reliable.

B. ON-LINE PRE-CONCENTRATION

This technique, also known as on-line or on-column trace enrichment, uses the same principles as SPE for the extraction of analytes from a water sample, but differs from the latter in that the solid sorbent is connected directly to the chromatographic instrument as implied by its name. As with SPE, a volume of sample (often several hundred mL) is passed through a solid sorbent. Usually a short pre-column (3 to 5 cm) is used as the solid phase sorbent. This approach is distinctively different from that of SPE, in which only a part of the concentrated eluate can be injected into the HPLC system. In the on-line pre-concentration method, the entire sample can be analyzed quantitatively. An appropriate mobile phase, introduced by a simple valve switch after the loading of sample, will elute essentially all of the concentrated analytes (20).

Kirkland was among the first to present the concept of on-line pre-concentration by suggesting that organic analytes could be concentrated on an analytical HPLC column (21). Little and Fallick (20), in 1975, investigated Kirkland's theory by pre-concentrating 200 mL of untreated sample water from a Massachusetts river on a Bondapack C-18 analytical column. The authors state that increased
sample recovery, and the lack of a phase change that could lead to re-arrangement or degradation of analytes when evaporated, as the advantages of the trace enrichment technique over other methods. This technique became known as on-column trace enrichment (22,23). As it is unwise to pass large volumes of water through an analytical HPLC column, pre-columns were soon used instead (13,24,25).

Ogan et al. (13) utilized a 10 cm 40 micron pellicular C-18 pre-column for the extraction of polycyclic aromatic hydrocarbons (PAH's) from aqueous samples. No mention is made of sample loading flow rates but sample volumes of up to 400 mL resulted in detection limits of 0.2 ng/mL. The authors reported the following advantages of the technique for the analysis of aqueous environmental samples.

1. The pre-column could be used for field sampling, thus reducing sample handling and possibly minimizing degradation.
2. Short pre-columns packed with large size particles allow high sample loading rates and reduced sampling time.
3. Most of the sample water and non-adsorbed compounds do not reach the analytical column.

Van Vliet et al. (25), used phthalate esters as model compounds for the investigation of on-line trace enrichment. The authors investigated several experimental parameters to optimize the trace enrichment process such as the size of pre-column sorbent, length of pre-column, sample volume, and sample loading rate. Their findings included:
1. A 20% decrease in analyte peak height when a 5 mm long x 2.9 mm internal diameter (i.d.) pre-column was used versus a 2 mm x 4.6 mm i.d. pre-column. The system backpressure was also substantially higher for the smaller bore column.

2. Replacement of a 5 micron pre-column packing with 10 micron packing resulted in a slight decrease in peak height. Larger than 50 micron packing resulted in poor performance.

3. There was no deterioration of efficiency in adsorption of the analytes when sample loading rates were increased to 25 mL/min from 5 mL/min. Recovery of the analytes was found to be quantitative in this range.

4. Sample volumes of up to 1000 mL could be passed through the pre-column.

5. Relative standard deviations for 10 sample runs were of the order of 5%.

6. For sample volumes of 300-1000 mL of untreated river water, clogging of the pre-column was 'a recurrent phenomenon'.

7. Band broadening is of the same order of magnitude as that obtained with a conventional 10-20 microliter injection.

These examples show that the recovery of compounds can be quantitative but that the pre-concentration apparatus must be optimized with respect to pre-column length, packing size, sample loading rate, and sample size.

With the exception of solid sorbent technology, the technique has changed little since these initial studies. Harvey and Stearns (22) provide an overview of different variations of valving schemes for on-line pre-concentration. The complexity of these valving
schemes depends upon the desired applications. On-column trace enrichment requires only one 2-position six port valve (22) as does a conventional pre-column system in which only unidirectional elution is required. Unidirectional elution involves the application of the mobile phase to the pre-column in the same direction in which the sample was loaded. More versatile valving systems enabling both unidirectional and backflush elution (application of the mobile phase to the pre-column in a direction opposite to that in which the sample was loaded) require two or more valves (22, 26). Other than the valving system, all that is required for the routine analysis of samples is a conventional HPLC solvent delivery system equipped with an ultra-violet (UV) or fluorescence detector.

Goewie et al. (27) studied the efficiency of the different pre-columns available and Lovkvist and Jonsson (28), and Werkoven-Goewie et al. (24) have studied sampling capacity of pre-columns. Goewie et al. (27) suggest that short pre-columns for on-line pre-concentration are preferable to longer ones. Longer pre-columns (30-40 mm) may have high efficiency but tend to deteriorate rapidly when exposed to large volumes of aqueous sample. Initially, the pre-columns were found not to have a negative influence on system efficiency, which is in agreement with Van Vliet et al. (25). However, the longer pre-columns were found to dramatically decrease system efficiency after 5 consecutive on-line pre-concentration experiments, suggesting that the pre-column packing had been distorted. Interestingly, no decrease in system efficiency was observed when backflush elution was used to elute the analytes from the longer pre-column. With shorter pre-columns (1.5 - 2 mm),
no decrease in system efficiency was observed after 6 to 8 sample runs. Unfortunately, these shorter pre-columns were home-made and not commercially available as are the longer pre-columns.

The authors have also included an overview of the considerations that must be addressed when choosing a pre-column for on-line analysis. These include:

1. The pre-column should display high retention for the analytes and have high capacity.
2. The pre-column should show negligible retention of the analytes during elution so that peak broadening is minimized.
3. The dead volume between the pre-column and the analytical column should be minimal.
4. The pre-column should not impose restrictions on sample loading rates due to high backpressures.
5. The pre-column should be simple to replace and inexpensive.

Pre-column sorbent technology now rivals that of SPE and offers the analyst the choice of a number of stationary phase compositions and sizes in commercially available pre-packed cartridges. C-18 sorbents are used most frequently for the analysis of water samples (29,30).

C. CARBAMATE PESTICIDES

The pesticides chosen for this study were selected as they are of concern in Ontario environmental water samples. The method currently used by the Ontario Ministry of the Environment for the analysis of carbamate residues in water involves liquid-liquid
extraction and subsequent reversed-phase HPLC analysis (1). This method provided a gradient elution program for the separation of analytes in samples prepared by the pre-concentration methods developed in the study.

Carbamate pesticides (31) are considered to be one of the three major classes of synthetic organic pesticides, the other two being the organochlorine (32) and organophosphorus (33) classes. Carbamates were extensively developed in the 1960's after the side effects of DDT (32) had been realized, although their potential as pesticides had been investigated as early as 1931 (34).

Carbamate pesticides can be generally described as carbamic acid esters (31).

\[
\text{HOCONH}_2 \quad \text{Carbamic Acid}
\]

The acid itself is very unstable and readily decarboxylates to form carbon dioxide and ammonia. However, the acid can be stabilized by conversion to the ethyl ester, also known as urethane.

\[
\text{C}_2\text{H}_5\text{OCONH}_2 \quad \text{Urethane}
\]

The phenyl N-methyl aryl ester of the acid has been found to be mildly toxic to several insect species (31).

\[
\text{PhOCONHCH}_3
\]
The addition or substitution of aryl and/or alkyl substituents on the phenyl ring or the amine group results in the formation of the pesticides used in this study.

Synthesis of most carbamates involves the esterification of a phenol with (A) methyl isocyanate, (B) phosgene and methylamine, or (C) methyl carbamoyl chloride (31).

(A) \[
\text{PhOH} + \text{CH}_3\text{NCO} \rightarrow \text{PhOCNHCH}_3
\]

(B) \[
\text{PhOH} + \text{ClCCl} \rightarrow \text{PhOCCl} + \text{NH}_2\text{CH}_3 \rightarrow \text{PhOCNCH}_3
\]

(C) \[
\text{PhOH} + \text{ClCNHCH}_3 \rightarrow \text{PhOCNHCH}_3
\]

Method A is the most widely used method for the synthesis of both aromatic and aliphatic N-methyl carbamates (31,35). The method can be modified for the synthesis of N-alkyl or N-aryl carbamates by substitution of appropriate reagents for methyl isocyanate (31). The reaction results in high yields and is quite exothermic (31). Method B is used less frequently (lower yields) but is preferred for some substituted phenol reagents (31). Method C is rarely used but is sometimes the method of choice for the synthesis of N,N-dimethylcarbamates, when N,N-dimethylcarbamoyl chloride is used (31,36).

Most carbamate pesticides are characterized by their high melting points, low vapour pressures, high solubilities in organic solvents, and low solubilities in water (31). Carbamates are prone to photodegradation, air oxidation, and slow decomposition in
aqueous media (31). This decomposition is hastened by increases in temperature or alkalinity (31).

Several mechanisms have been proposed for the hydrolysis of N-methyl carbamates. These are illustrated in Fig. 1 (37, 38). Substitution of the N-methyl group on the nitrogen of N-methyl carbamates with larger alkyl groups decreases resistance to hydrolysis (35). The addition of electron withdrawing substituents to the ring of aromatic N-methyl carbamates also decreases resistance to hydrolysis (35). Di-substitution of the nitrogen increases resistance to hydrolysis (35). These effects are summarized in Table 1. Hydrolysis of carbamates also occurs in acidic media, but at a rate much slower than that in alkaline media (31, 39).

A listing of the carbamate pesticides used in this study can be found in Appendix 1. The listing includes alternative names, history and physical properties, solubility in water, and the acute oral toxicities for rats (40).

Unlike the organochlorine class of pesticides, carbamates generally do not lend themselves easily to analysis by GC. This is mainly due to thermal lability (31). As a result, HPLC is widely used for the determination of carbamate pesticides.

Methods involving GC analysis have been described (41,42). Colorimetric (43,44), enzymic (45,46), spectrofluorimetric (47, 48), and mass spectral (49, 50) techniques for the determination of carbamates and their metabolic derivatives in various sample matrices have been described in the literature. However, each of the aforementioned methods has limitations making it inappropriate for
Fig. 1  Proposed mechanisms for the hydrolysis of N-methyl carbamates \(^{(31, 37)}\).

A  \[ R\overset{O}{C}\overset{O}{N}(CH_3)_2 + OH^- \rightleftharpoons [R\overset{O}{C}\overset{O}{N}(CH_3)_2] \rightarrow R\overset{O}{OH} + (CH_3)_2NCO^- \]
\[ (CH_3)_2NCO^- + H_2O \rightleftharpoons (CH_3)_2NCOH + OH^- \]
\[ (CH_3)_2NCOH \rightarrow (CH_3)_2NH + CO_2 \]

B  \[ Ar\overset{O}{C}\overset{O}{NH}CH_3 + OH^- \rightleftharpoons [Ar\overset{O}{C}\overset{O}{N}CH_3] + H_2O \rightarrow ArO^- + CH_3NCO \]
\[ [Ar\overset{O}{C}=NCH_3] \]
\[ CH_3NCO + H_2O \rightarrow CH_3HNCOH \]
\[ CH_3HNCOH \rightarrow CH_3NH_2 + CO_2 \]
Table 1. Second order rate constants for the hydrolysis of substituted phenyl N-methylcarbamates and phenyl N-substituted carbamates. The rate constant units are mole\(^{-1}\)min\(^{-1}\). The rate constants for the substituted phenyl N-methylcarbamates and the phenyl N-substituted carbamates were measured in barbital buffer, 0.1 M, pH 9.5, 37.5\(^\circ\)C. The rate constants for the phenyl N,N-disubstituted carbamates were measured in phosphate buffer, 0.05 M, pH 7.8, 22 \(^\circ\)C (31,35).

<table>
<thead>
<tr>
<th>Substituted Phenyl N-methylcarbamate</th>
<th>(K_{hyd})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-NO(_2)</td>
<td>(3.4 \times 10^6)</td>
</tr>
<tr>
<td>4-NO(_2)</td>
<td>(3.5 \times 10^5)</td>
</tr>
<tr>
<td>2-Cl</td>
<td>(2.0 \times 10^3)</td>
</tr>
<tr>
<td>3-Cl</td>
<td>(1.7 \times 10^3)</td>
</tr>
<tr>
<td>4-Cl</td>
<td>(1.0 \times 10^3)</td>
</tr>
<tr>
<td>3-CH(_3)</td>
<td>(3.0 \times 10^2)</td>
</tr>
<tr>
<td>2-CH(_3)</td>
<td>(2.6 \times 10^2)</td>
</tr>
<tr>
<td>2-i-C(_3)H(_7)</td>
<td>(5.5 \times 10)</td>
</tr>
<tr>
<td>2-t-C(_4)H(_9)</td>
<td>(2.8 \times 10)</td>
</tr>
<tr>
<td>3-N(CH(_3))(_2)</td>
<td>(2.0 \times 10)</td>
</tr>
<tr>
<td>3-t-C(_4)H(_9)</td>
<td>(0.4 \times 10)</td>
</tr>
</tbody>
</table>

Phenyl N-substituted Carbamates

<table>
<thead>
<tr>
<th></th>
<th>(K_{hyd})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHC(_6)H(_5)</td>
<td>(5.8 \times 10^3)</td>
</tr>
<tr>
<td>NHCH(_2)C(_6)H(_5)</td>
<td>(8.2 \times 10^2)</td>
</tr>
<tr>
<td>NHC(_2)H(_5)</td>
<td>(5.0 \times 10^2)</td>
</tr>
<tr>
<td>NHCH(_3)</td>
<td>(2.5 \times 10^2)</td>
</tr>
</tbody>
</table>

Phenyl N,N-disubstituted Carbamates

<table>
<thead>
<tr>
<th></th>
<th>(K_{hyd})</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N-diCH(_3)</td>
<td>(3.9 \times 10^{-3})</td>
</tr>
<tr>
<td>N,N-diC(_2)H(_5)</td>
<td>(6.2 \times 10^{-5})</td>
</tr>
</tbody>
</table>
the analysis of large quantities of aqueous solution containing pesticide residues at the ppb or ppt levels. As a result, HPLC is generally regarded to be the best technique for carbamate residue analysis in water. Several authors have described SPE (51, 52) and on-line pre-concentration techniques (53) in conjunction with HPLC for the analysis of carbamate pesticides residues in water. EPA method 531 (54) uses HPLC for the determination of carbamates.

Wolkoff and Creed (51) compared the techniques of on-column trace enrichment using a Radial-Pak A analytical column, and SPE using Waters Sep-Pak C-18 cartridges. Trace enrichment of four carbamate pesticides (Carbofuran, Carbaryl, Propham, and Chlorpropham, ranging in concentration from 39 ppb for Carbaryl to 200 ppb for Propham) was performed by passing 45 mL of sample through a Radial-Pak A column and then switching to an acetonitrile/water gradient elution program. The analytes were determined by UV absorption at 254 nm. The Sep-Pak cartridges were wetted with 5 mL of acetonitrile or methanol and then flushed with 5 mL of water in preparation for the application of 45 mL of sample water at 10 mL/min. The analytes were then eluted with 2 mL of THF. The effluent from the sample loading step was trace-enriched to determine if any breakthrough was apparent. None was observed. Interesting results were obtained from the analysis of an actual environmental sample. A sample collected and shipped in glass and analysed by the trace enrichment method was compared with a sample pre-concentrated on a Sep-Pak C-18 cartridge on-site. Although more early eluting material was observed in the trace enrichment chromatogram (probably due to the greater
capacity of the Radial-Pak A column versus the Sep-Pak), far more late eluting components are seen in the chromatogram resulting from the analysis of the sample collected on-site using the Sep-Pak. The authors suggested that this is due to compounds being adsorbed onto the glass container used in the shipping of the sample analyzed by the trace enrichment technique. They also concluded that the SPE cartridges could be 'simple and efficient devices for on-site sampling'. This would result in much lower sample transportation costs.

Chaput (53) employed a 3.7 cm x 4.6 mm i.d. 10 micron C-8 Brownlee pre-column installed in the loop position of a six-port valve for the pre-concentration of 10 mL samples containing Aldicarb, Aldicarb sulfoxide, and Aldicarb sulfone. The pre-concentrated analytes were backflushed to the analytical column by a methanol/water gradient and determined by fluorescence detection after a post-column hydrolysis step. Sampling flow rates were 10 mL/min, detection limits were 70 ng/L for the three compounds, and the recoveries of the compounds from the sample ranged from 71 to 78%. The author also used the technique for the analysis of untreated ground water samples. Calcium (40-50 mg/L) and nitrate (3-15 mg/L) in these samples had no adverse effects upon the recovery of analytes. These chromatograms are characterized by broad, early eluting peaks. This paper shows that quite polar N-methyl carbamates can be partitioned satisfactorily on reversed-phase material.

Bushway (52) compared the techniques of direct HPLC injection and SPE using Carbaryl and 1-naphthol as model
compounds. Carbaryl and 1-naphthol were determined at concentrations as low as 3.78 and 10 ppb respectively by the direct injection of 230 microliters into the HPLC. The analytes were separated isocratically (acetonitrile/water) on a C-18 analytical column and determined by UV absorption at 220 nm. The samples were pre-filtered through a 0.45 micron filter. The SPE cartridges (Waters Sep-Pak C-18) were wetted with 4 mL of methanol and then flushed with 5 mL of water. The analytes were eluted with 2 mL of acetonitrile. Of the 2 mL of eluate, a 50 microliter aliquot was injected into the HPLC. The sample loading rate was 22.2 mL/min for a 1000 mL sample. Detection limits were reported to be 0.099 ppb for carbaryl and 0.516 ppb for 1-naphthol. Salt water and stream water samples were also analyzed by this method, with both chromatograms showing large early eluting peaks. Recovery of the analytes was near 100%. The capacities of the SPE cartridges were determined to be approximately 1.5 micrograms for 1-naphthol and greater than 4.5 micrograms for Carbaryl. The author also found that, under the described operating conditions, each SPE cartridge could be used for three analyses.

D. BENOMYL

Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) is used worldwide as a systemic fungicide for disease control in crops. The analysis of benomyl residues in water is made difficult by the instability of the compound in different organic solvents (55,56,57) and by its very low solubility in water (58).
These characteristics distinguish Benomyl from other carbamate pesticides. Benomyl also decomposes in water, but at a rate slower than that in organic solvent (59,60).

High-performance liquid chromatographic (HPLC) methods are most popular for the analysis of benomyl but most employ the determination of the degradation product, methyl 2-benzimidazolecarbamate (Carbendazim or MBC) after quantitative conversion of the parent compound (61,62,63,64,65).

Kirkland et al .(62) converted Benomyl residues in soil and plants to MBC by treatment with acid. The resulting MBC was analyzed by HPLC using a cation exchange column. Zweig and Gao (63) used acetonitrile both for extraction of Benomyl from samples and for quantitative conversion of Benomyl to MBC in a short period of time (a prescribed waiting period of 3 hours at room temperature or 1 hour at 40 C to ensure 100% conversion of Benomyl to MBC). Benomyl was determined as MBC by isocratic elution (50% acetonitrile/50% water) on a reversed-phase HPLC column. Spittler et al .(64) have described a technique whereby both MBC and another Benomyl metabolite, 2-aminobenzimidazole (2-AB), are isolated from various sample matrices and determined by cation-exchange LC.

These techniques are unsatisfactory in that the MBC that is produced from the parent benomyl during the sample preparation procedure cannot be distinguished from MBC that was present in the sample as a natural degradation product of benomyl. This methodology, which is not acceptable in principle, has been widely used in the past, however, for the following two reasons. The main reason is that the determination of intact Benomyl residues is
exceptionally difficult. Another reason is that MBC is also fungitoxic, and the fungitoxicity of Benomyl is, in fact, thought to be due to the presence of MBC (66).

An HPLC method for the simultaneous determination of benomyl and MBC in aqueous media has been described (67). Benomyl is quantitatively converted by treatment with base to 3-butyl-2,4-dioxo-s-triazino[1,2-a] benzimidazole (STB) while MBC present in the sample matrix is unaffected and determined as MBC. This technique is suitable for the analysis of benomyl and MBC at the low parts per million (ppm) level.

For reasons discussed earlier, it would be advantageous to minimize exposure of the sample to any organic solvent during the sample preparation procedure. This prevents the application of SPE to the analysis of Benomyl residues. On-line pre-concentration (or trace enrichment) offers the possibility of isolating intact Benomyl directly from an aqueous sample matrix by retaining it on the solid sorbent contained in a short pre-column. A subsequent valve switch allows mobile phase to flush Benomyl from the pre-column to the HPLC without further sample manipulation. A buffered mobile phase must be employed to produce an MBC peak that is sharp enough for accurate quantitation. This modification of the on-line pre-concentration technique should enable simultaneous determination of Benomyl, MBC, and the other carbamate pesticides previously introduced.

E. SAMPLE PREPARATION AUTOMATION
One of the goals in analytical methods development is to make the preparation of a sample as free as possible from time consuming steps and/or manual manipulation. It has been said that sample preparation is the weak link in chemical analysis (68). This has led to the development of 'automated' methods and the implementation of robotics into existing laboratory instrumentation. Laboratory automation should result in less variability in results and decrease the risk of error or contamination.

On-line pre-concentration techniques should lend themselves more easily to automation than SPE methods in which external sample collection and separate injection into an instrument for analysis were essential. Some on-line pre-concentration techniques require sample or valve switching as the only manual step in the replicate analysis of samples (53,69). The technique can be fully automated by micro-processor control of pneumatically activated switching valves (26,70). The use of multi-port sampling valves results in the ability to analyse many samples consecutively and unattended. Ramsteiner (70) has developed this technique for the analysis of organic compounds in ground and tap waters. Two 16-port sampling valves are connected to a trace enrichment system employing two more 8-port valves. This system allows for the flushing of the sample inlet connecting tubes and the application of a low polarity rinsing solvent to the reversed-phase enrichment column in an attempt to eliminate potential interferences.

SPE has proven to be a more difficult technique to automate, being an off-line technique and frequently requiring evaporation and dilution steps to maximize sensitivity. Laboratory robotics systems
have been used to automate some of the SPE processes including the addition of internal standards, prewetting or 'conditioning' of the SPE cartridge, sample loading, analyte elution, and sample transfer to an autosampler (71). Unfortunately, these systems are frequently limited to the preparation of samples of volumes of 10 mL or less. Laboratory robotics systems are known to be cost effective as alternatives to humans performing menial and repetitious tasks (72). However, the price range of a basic robotics system is much more than that of instruments designed specifically to perform SPE.

Yago (73), has described an advanced automated sample processor (AASP) marketed by Varian Associates for 'automated' SPE. The AASP is the first example of a commercially marketed instrument specifically designed for SPE. The features of the AASP include the ability to automatically pass sample through the SPE cartridge under vacuum pressure and the ability to transfer analytes directly from the cartridge onto the head of an HPLC column by a valve switch. The latter point reflects a relation to trace enrichment as the elution of the analytes is performed on-line. Elution of the analytes from the cartridge is quite rapid as the Varian SPE cartridges contain only 50 mg of solid sorbent. The ability to pass sample through the cartridge under positive pressure is more desirable than using a simple vacuum manifold in that the flow rate of liquid through the SPE cartridge can be more accurately controlled with positive pressure than with vacuum (negative) pressure. As mentioned in the introductory section to SPE, accurate control of the flow rate through the cartridge of sample and elution solvent is pivotal to obtain reproducible results.
The major drawback of the AASP is the fact that manual manipulation of the cartridges is necessary. The SPE cartridges must be transferred from the sample loading manifold to the AASP itself by hand. The sample loading module is quite compact and could be portable for field samples. The small amount of sorbent contained in the cartridge may be a drawback when working with large or concentrated sample volumes requiring large sorbent capacities. Furthermore, the AASP does not allow for dilution of the eluting solvent with water before injection into the HPLC. This can result in poor analyte peak profiles, especially when a mobile phase used is a weak one containing a high percentage of water (74).

Fallick (75) described the Water Millilab Workstation in 1987. This instrument offers the possibility of totally automated SPE including the cartridge conditioning, sample loading, analyte elution, eluate blow-down, sample dilution, and sample injection steps. Sample and solvent are delivered to the SPE cartridge by a syringe pump so that accurate control of the flow rate is possible. By use of an X-Y robotic arm, the eluate from the cartridge can be collected and evaporated with an external gas supply connected to the instrument through a rotary valve. The sample can then be diluted with water, aspirated into the robotic arm probe, and injected directly into the HPLC. A microprocessor can control gradient elution of the analytes and data acquisition. With the aid of additional sampling valves, the Millilab can analyse many samples consecutively and unattended. The Millilab is the backbone of the method developed for the automated analysis of carbamate pesticide residues in water using SPE that is described in this work.
E. SCOPE OF THE STUDY

The aim of the study was to compare the two techniques previously introduced, SPE and on-line pre-concentration. The methods were evaluated on the basis of their ability to determine trace concentrations of carbamate pesticides in water. On-line pre-concentration was performed by the addition of a valving system to a conventional HPLC instrument. SPE was performed using an X-Y robotic sample preparation instrument specifically designed by a major instrument manufacturer for this purpose.

Many factors had to be considered in the evaluation of the methods. These included sensitivity, reproducibility, time per sample analysis, and cost per sample analysis. Attempts were also made to fully automate each of the procedures. Automation presents unique problems for each of the two procedures, as discussed previously.

The introductory sections on SPE and on-line pre-concentration present not only the historical development of the two methods, but also an overview of the experimental parameters that must be studied to optimize the methods. Some of these parameters are common to both methods, such as sample flow rate through the solid sorbent, and sorbent particle diameter. The references cited in the introductory sections proved invaluable in that they provided experimental information to be used in setting up initial experimental procedures that could then be further optimized.
Among the experimental parameters investigated in the on-line pre-concentration study were 1) size of packing material used in the pre-column 2) the rate of sample loading onto the pre-column 3) properties of the solid sorbent phase 4) pre-column longevity 5) cost of operation 6) whether backflushing of the pre-column is required 7) type of analytical column and 8) minimum detectable concentrations.

The parameters investigated in the SPE study included 1) the flow rate of sample through the SPE cartridge 2) the amount of sorbent needed in the cartridge to adequately retain the analytes 3) sorbent packing material diameter 4) cost of operation 5) minimum detectable concentrations 6) volume of eluting solvent 7) final volume of eluate after the blow-down step under nitrogen and 8) the water content of the sample before injection into the HPLC.

Additional information about SPE was obtained from the results of a study involving the analysis of chlorophenols and phenoxy-acids in water. The study focussed predominantly on the effect of varying solvent composition and polarity on analyte recovery, but additional information regarding sorbent, analyte, and mobile phase interactions was obtained. The results of this study are contained in Appendix 2.

As seen in the introduction, a separate section of text is devoted entirely to the development of a method for the determination of Benomyl. The analysis of Benomyl presents unique problems and these were addressed after the development of SPE and on-line pre-concentration procedures for the other pesticides. The on-line pre-concentration method developed for the other pesticides
was modified, predominantly by the substitution of a buffered mobile phase, to allow for the determination of both Benomyl and its degradation product, Carbendazim (MBC).
EXPERIMENTAL SECTION

A. ON-LINE PRE-CONCENTRATION

Solvents

Acetonitrile was of HPLC grade from Fisher Scientific (Fairlawn, NJ), and Caledon Laboratories (Georgetown, Ontario, Canada). Water used for preparation of standards was distilled in glass in the laboratory.

Pesticides

Solid pesticide standards were obtained from the United States Environmental Protection Agency, Research Triangle Park, NC. Purities of the individual standards ranged from 97.5 to 100%. The pesticides, listed in the order in which they appear in the chromatograms, are 1) Propoxur, 2) Carbofuran, 3) Carbaryl 4) Propham, 5) Captan, 6) Chloropropham, 7) Barban, and 8) Butylate.

Preparation of Stock Standard Solutions

Solid standards were dissolved in acetonitrile and diluted in acetonitrile. These individual stock standard solutions were combined at different concentrations because of varying sensitivities to ultra-violet (UV) detection. The combined standard solution, thus prepared, was diluted with water to make standard water samples as below.

Water Samples

Standard water samples were prepared by diluting 1 mL of the combined standard solution (prepared as above) to 1000 mL with
distilled water from the laboratory unless otherwise noted. The following types of water samples were investigated: municipal tap waters, distilled waters, commercial HPLC grade waters, commercial spring drinking waters, reverse osmosis water, and ion exchange water.

**HPLC Apparatus**

The HPLC system consisted of a Waters model 510 pump, a Waters model 501 pump, a Waters WISP model 710B sample processor, a Waters model 484 tunable absorbance UV detector, a Fisher Recordall series 5000 strip chart recorder, and a Digital Professional 350 computer system (Digital Equipment Corp., Maynard, MA) incorporating Waters 840 chromatography software (Waters Assoc., Millford, MA). A Waters model 600 Powerline solvent delivery system was used in additional sample loading rate experiments.

Pre-columns were 5 micron Spherisorb C-18 and C-8 3 cm x 4.6 mm i.d. cartridges from Brownlee Labs (Santa Clara, CA) and 3 cm x 4.6 mm i.d. laboratory packed with 10 micron Vydac Reverse Phase TP-201 (The Separations Group, Hesperia, CA), 10 micron Ultrasil ODS (Altex Scientific, Berkely, CA), and 40 micron Whatman Co-Pell ODS (Whatman Ltd., Clifton, NJ). Analytical columns were a Supelcosil LC-8 5 micron 25 cm x 4.6 mm i.d. (Supelco Inc., Bellefonte, PA), and a Phenomenex Spherisorb C-18 5 micron 15 cm x 4.6 mm i.d. (Phenomenex Inc., Torrance, CA).

The on-line pre-concentration apparatus (Fig 12) incorporated 2 high pressure in-line filters with 0.5 micron frits from Mandel Scientific (Guelph, Ontario, Canada), and 3 Rheodyne model 7000 2
position 6 port switching valves, one of which was equipped with a model 5701 air actuator controlled by a Rheodyne model 7163 solenoid valve kit (Rheodyne Inc., Cotati, CA).

**Operating Conditions**

Wavelength, 220 nm; flow rate, 1.5 mL/min; chart speed, 0.5 cm/min; detector sensitivity, 0.075 AUFS (1 mV = 1 x 10^{-3} AU); recorder range, 10 mVFS; column temperature, ambient.

**On-line pre-concentration**

100 mL of water sample was passed through the pre-column at 5 mL/min while the apparatus was in the 'load' position.

**Elution**

The following gradient program for the C-8 analytical column was run after switching the valves to the 'elute' position from the 'load' position:

<table>
<thead>
<tr>
<th>Elapsed time</th>
<th>Composition of mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>5 min</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>15 min</td>
<td>60% acetonitrile, 40% water</td>
</tr>
<tr>
<td>25 min</td>
<td>60% acetonitrile, 40% water</td>
</tr>
<tr>
<td>30 min</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>35 min</td>
<td>30% acetonitrile, 70% water</td>
</tr>
</tbody>
</table>

Changes in the percentage of solvents in the mobile phase throughout the gradient program occurred linearly. The final 10 minutes of the gradient program serves to return the system to the initial conditions to enable another analysis run.
A gradient program was also developed for a C-18 analytical column;

<table>
<thead>
<tr>
<th>Elapsed time</th>
<th>Composition of mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>5 min</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>10 min</td>
<td>60% acetonitrile, 40% water</td>
</tr>
<tr>
<td>20 min</td>
<td>70% acetonitrile, 30% water</td>
</tr>
<tr>
<td>25 min</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>30 min</td>
<td>30% acetonitrile, 70% water</td>
</tr>
</tbody>
</table>

Changes in the percentage of solvents in the mobile phase throughout the gradient program occurred linearly.
B. SOLID PHASE EXTRACTION

Solvents

Acetonitrile was of HPLC grade from Fisher Scientific (Fairlawn, NJ), and Caledon Laboratories (Georgetown, Ontario, Canada). Water used for preparation of standards was distilled in glass in the laboratory. HPLC grade water from Fisher Scientific (Fairlawn, NJ) was used to provide the cleanest possible chromatograms.

Pesticides

Solid pesticide standards were obtained from the United States Environmental Protection Agency, Research Triangle Park, NC. Purities of the individual standards ranged from 97.5 to 100%. The pesticides, listed in the order in which they appear in the chromatograms, are 1) Propoxur, 2) Carbofuran, 3) Carbaryl 4) Propham, 5) Captan, 6) Chloropropham, 7) Barban, and 8) Butylate.

Preparation of Standard Solutions

Solid standards were dissolved in acetonitrile and diluted in acetonitrile. These individual standard solutions were combined at different concentrations because of varying sensitivities to ultraviolet (UV) detection. The combined standard concentration of each pesticide is listed in Table 13.

Water Samples

Standard water samples were prepared by adding 1 mL of the combined standard to 1000 mL of distilled water from the laboratory unless otherwise noted. Several tap and untreated surface waters were collected from local sources and analysed.
HPLC Apparatus

The HPLC system consisted of a Waters Millilab Workstation, a Waters 600E Powerline Multisolvent Delivery System, a Waters 484 tunable absorbance UV detector, four Waters Multiple Intake Accessories (for use with the Millilab), and a Waters 815 or 840 Baseline chromatography software package (Waters Assoc., Millford, MA).

SPE Cartridges and Analytical Column

Three types of SPE cartridges were evaluated in the study; 1) the Waters Sep-Pak Plus C-18 cartridges containing 330 mg of 90 micron sorbent 2) the Waters Sep-Pak Light C-18 cartridges containing 120 mg of 90 micron sorbent and 3) the Waters Sep-Pak custom C-18 cartridges containing 330 mg of 40 micron sorbent (Waters Assoc., Millford, MA).

The analytical column was a Supelcosil LC-8 5 micron 25 cm X 4.6 mm i.d. (Supelco Inc., Bellefonte, PA).

HPLC Operating Conditions

Wavelength, 220 nm; flow rate, 1.5 mL/min; chart speed, 0.5 cm/min; detector sensitivity, 0.075 AUFS; recorder range, 10 mVFS; column temperature, ambient.

Gradient Elution Program

<table>
<thead>
<tr>
<th>Elapsed Time</th>
<th>Composition of mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>5 min.</td>
<td>30% acetonitrile, 70% water</td>
</tr>
<tr>
<td>15 min.</td>
<td>60% acetonitrile, 40% water</td>
</tr>
<tr>
<td>25 min.</td>
<td>60% acetonitrile, 40% water</td>
</tr>
<tr>
<td>30 min.</td>
<td>30% acetonitrile, 70% water</td>
</tr>
</tbody>
</table>
Changes in the percentage of solvents in the mobile phase throughout the gradient program occurred linearly. The final 10 minutes of the gradient program serves to return the system to the initial conditions to enable another analysis run.

**SPE Procedure**

1. Pass 1 mL of acetonitrile through a Sep-Pak Light cartridge as a conditioning step.
2. Pass 2.5 mL of water through the cartridge in preparation for sample loading.
3. Pass 100 mL of sample through the cartridge at a flow rate of 10 mL/min.
4. Air dry the cartridge for a period of 15 seconds.
5. Elute the analytes from the cartridge by passing 0.750 mL (3 X 0.250 mL) of acetonitrile through the cartridge at 2.0 mL/min.
6. Gently blow-down the eluate with nitrogen at 5 psi for 15 minutes to 0.20 mL± 0.01 mL.
7. Make up the sample solution to 0.35 mL with water.
8. Inject 0.175 mL of the prepared solution into the HPLC via the Millilab.

Note: The prepared samples should be analysed as soon as possible after preparation as the concentrated Captan is very insoluble in aqueous media (solubility in water is approximately 0.5 ppm) and adsorbs readily on glass.
C. BENOMYL

Solvents

Acetonitrile was of HPLC grade from Fisher Scientific (Fairlawn, NJ), and Caledon Laboratories (Georgetown, Ontario, Canada). Water used for preparation of standards was distilled in glass in the laboratory.

Pesticides

Benomyl was purchased commercially as Benlate wettable powder (Wilson Laboratories, Laval, P.Q., 50% active ingredient). Using the method developed by Chiba and Singh (67), the active ingredient in the Benlate powder was determined to be 54.5%, of which 86% was Benomyl and 14% MBC. Other solid pesticide standards were obtained from the United States Environmental Protection Agency, Research Triangle Park, NC. Purities of the individual standards ranged from 97.5 to 100%. The pesticides, listed in the order in which they appear in the chromatograms, are 1) MBC, 2) Aminocarb, 3) Propoxur, 4) Carbofuran, 5) Carbaryl 6) Propham, 7) Captan, 8) Chloropropham, 9) Barban, 10) Benomyl, and 11) Butylate.

Preparation of Stock Standard Solutions

Solid standards (with the exception of Benomyl and MBC) were dissolved in acetonitrile and diluted in acetonitrile. MBC was dissolved in methanol and diluted in methanol while Benomyl was prepared as a suspension in distilled water.

As Benomyl solutions decompose at room temperature (76), Benomyl standard solutions should be refrigerated. Benomyl
standard suspensions containing Benomyl at concentrations greater than its solubility in water must be thoroughly stirred before dilution to ensure an even distribution of particulate matter in any aliquot removed. Even freshly prepared Benomyl standards were observed to contain some MBC.

The individual stock standard solutions were combined at different concentrations because of varying sensitivities to ultraviolet (UV) detection. The combined standard solution thus prepared was diluted with water to make standard water samples as below.

**Water Samples**

Standard water samples were prepared by diluting 1 mL of the combined standard solution (prepared as above) to 1000 mL with distilled water from the laboratory unless otherwise noted.

**HPLC Apparatus**

The HPLC system consisted of a Waters model 600 Powerline solvent delivery system, a Waters WISP model 710B sample processor, a Waters model 484 tunable absorbance UV detector, a Fisher Recordall series 5000 strip chart recorder, and an NEC Powermate 2 computer system (NEC Information Systems Inc., Boxborough, MA) incorporating Waters 810 chromatography software (Waters Assoc., Millford, MA).

Pre-columns were 5 micron Spherisorb C-18 and C-8 3 cm x 4.6 mm i.d. cartridges from Brownlee Labs (Santa Clara, CA). Analytical columns were a Supelcosil LC-8 5 micron 25 cm x 4.6 mm i.d. (Supelco Inc., Bellefonte, PA), and a Phenomenex Spherisorb C-18 5 micron 15 cm x 4.6 mm i.d. (Phenomenex Inc., Torrance, CA).
The on-line pre-concentration apparatus (Fig. 12) incorporated 2 high pressure in-line filters with 0.5 micron frits from Mandel Scientific (Guelph, Ontario, Canada), and 3 Rheodyne model 7000 2 position 6 port switching valves, one of which was equipped with a Rheodyne model 5701 air actuator controlled by a Rheodyne model 7163 solenoid valve kit (Rheodyne Inc., Cotati, CA).

**HPLC Operating Conditions**

Wavelength, 220 nm; flow rate, 1.5 mL/min; chart speed, 0.5 cm/min; detector sensitivity, 0.075 AUFS (1 mV = 1 x 10^{-3} AU); recorder range, 10 mV.F.S.; column temperature, ambient.

**On-line pre-concentration**

100 mL of water sample was passed through the pre-column at 5 mL/min while the apparatus was in the 'load' position unless otherwise noted.

**Elution**

The following gradient program was run after switching the valves to the 'elute' position from the 'load' position:

<table>
<thead>
<tr>
<th>Elapsed time</th>
<th>Composition of mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>30% acetonitrile, 70% buffer</td>
</tr>
<tr>
<td>5 min.</td>
<td>30% acetonitrile, 70% buffer</td>
</tr>
<tr>
<td>15 min.</td>
<td>60% acetonitrile, 30% buffer, 10% water</td>
</tr>
<tr>
<td>25 min.</td>
<td>60% acetonitrile, 30% buffer, 10% water</td>
</tr>
<tr>
<td>30 min.</td>
<td>30% acetonitrile, 50% buffer, 20% water</td>
</tr>
<tr>
<td>35 min.</td>
<td>30% acetonitrile, 70% buffer</td>
</tr>
</tbody>
</table>
Changes in the percentage of solvents in the mobile phase throughout the gradient program occurred linearly. The final 10 minutes of the gradient program serves to return the system to the initial conditions to enable another analysis run.

The inclusion of water in the mobile phase, resulting in a ternary gradient, was essential in order to maintain a flat baseline profile throughout the gradient program. Decreasing the buffer strength in the aqueous phase during increases in the percentage of acetonitrile, results in an optimum baseline profile. If water was not included in the mobile phase, thereby resulting in a binary gradient, a marked disturbance in the baseline (a huge bump) was unavoidable. The baseline disturbance can, however, be negated through the use of gradient correction.

**Preparation of Buffered Mobile Phase**

The 0.0035 M phosphate buffer (pH approximately 6.8) was prepared using potassium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate dihydrate (BDH Chemicals Ltd., Poole, England). This buffer was used as shown in the gradient elution program.
RESULTS AND DISCUSSION

A. PRELIMINARY STUDIES

A preliminary study of the carbamate pesticides was undertaken to observe the behaviour of the individual analytes under varying chromatographic conditions.

The first phase of the study involved optimization of the gradient elution program in an attempt to achieve maximum separation of the pesticides which were considered important by the Ontario Ministry of the Environment in 1987. These pesticides were prepared as a mixed concentrated standard solution (MOE 1, Appendix 4). A gradient program for the separation of the pesticides on a 25 cm C-8 analytical column (Ref. 1 and experimental section) was found to be adequate for the separation of most compounds with the exception of Butylate and Diallate. Therefore, the effects of altering the existing gradient were investigated using a 15 cm C-18 analytical column. The results were essentially the same as those obtained with the C-8 analytical column. All compounds could be separated with baseline resolution with the exception of Butylate and Diallate, two relatively non-polar analytes as exhibited by their long retention times (approximately 22 min). The different gradient programs investigated are illustrated in Figures 2 through 5.

Several general statements can be made regarding the gradient elution program. The initial composition of the mobile phase (30%
Fig. 2. Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1.
Fig. 3. Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1.
Fig. 4. Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1.

The optimum gradient for a C-18 analytical column is marked with an asterisk.
Fig. 5. Graph of different gradients investigated with a C-18 analytical column for the optimization of separation of analytes in MOE 1.
acetonitrile/70% water) must be maintained for a period of at least 5 minutes. Failure to do this results in poor resolution of the early eluting components (Propoxur and Carbofuran). The percentage of acetonitrile in the mobile phase must then be increased linearly as rapidly as possible to achieve maximum resolution of Butylate and Diallate, but not so rapidly as to result in poor separation of the intermediate eluting compounds. Fig. 6 is a chromatogram showing the best separation of the analytes contained in MOE 1. The optimum gradient program for the separation of analytes on a C-18 analytical column is outlined in the experimental section.

Benomyl is included in the listing of the pesticides making up MOE 1 but does not appear in the chromatogram (Fig. 6). In this case, the concentrated Benomyl standard was prepared in acetonitrile, resulting in the rapid decomposition of Benomyl to its major metabolic derivative, MBC. MBC was observed as a broad, early eluting peak in chromatograms produced by injecting a solution prepared as a Benomyl standard. The instability of Benomyl in organic solvents was unknown to the author at the time.

Splitting of the Butylate and Diallate components into three peaks was also observed during the gradient optimization investigation. It was confirmed that Diallate is indeed a mixture of two isomers, both by literature description of the compound, and by isocratic elution of the compounds by a mobile phase consisting of 60% acetonitrile/40% water. (Fig. 7).

Determination of the analytes was by UV absorption at 220 nm. A UV scan of the the concentrated standard (MOE 1) shows that 220 nm is near the absorption maximum for the mixture (Fig. 8).
Fig. 6. Chromatogram showing the best separation of the analytes in MOE 1 obtained from an 80 microliter injection of MOE 1 diluted approximately 10:1 in water. The chromatogram was produced using the developed C-18 gradient program and plotted at 300 millivolts.
Fig. 7. Chromatogram showing the results obtained from a 20 microliter injection of approximately 10 ppm of Diallate and 10 ppm Butylate in water. The analytes were separated on a C-18 analytical column isocratically (70% acetonitrile/30% water) at a flow rate of 1 mL/min. The chromatogram was plotted at 50 millivolts.
Fig. 8. Ultra-violet scan of MOE 1 diluted approximately 200:1 in water.

Operator: 
Date: 
Trace: 1
Sample identity: 
Absorbance mode 
Ordinate Maximum 0.800 
Ordinate Minimum 0.000 
Upper Wavelength 350.0 nm.
Lower Wavelength 190.0 nm. 
Scan Speed 100.0 nm/min.
Bandwidth 1.0 nm.
Time Constant 0.3 sec
2 Peak(s) detected
Peak at wavelength 310.6 nm. of value 0.000
Peak at wavelength 206.3 nm. of value 0.533
Deterioration of the solvent blank was also observed during the course of the study by the observation of late eluting peaks in the chromatograms. It was confirmed that this deterioration was due to contact of solvent vapours with rubber stoppers affixed to the top of the flasks containing the solvents making up the mobile phase (Fig. 9).

Comparison of the separation of the analytes on a C-8 analytical column using the gradient outlined in the experimental section shows better separation of the early eluting compounds than on the C-18 analytical column, but poorer resolution of the Butylate and Diallate (Fig. 10). Running the gradient program designed for the C-18 column on the C-8 column results in good separation of the early and intermediate eluting compounds but Butylate and Diallate co-elute (Fig. 11).

Initial Experiments with Carbaryl

Carbaryl was chosen as a model compound for the initial pre-concentration experiments as it exhibits good sensitivity to UV detection. L.K. She has reported an on-line pre-concentration method for the determination of Carbaryl using LC in conjunction with fluorescence detection (77). Four injections of incremental volumes of a concentrated Carbaryl standard were made to check for peak area linearity of detector response and to obtain an estimate of the peak areas to be expected from actual pre-concentration sample runs. These data are shown in Table 2. Before the sample loading step, the entire system including the C-8 analytical column was
Fig. 9. Chromatogram resulting from the analysis of an 80 microliter aliquot of an acetonitrile solution in which a rubber stopper was immersed for 12 hours. The chromatogram was produced using a C-18 analytical column with the developed C-18 gradient program. The chromatogram was plotted at 300 millivolts.
Fig. 10. Chromatogram resulting from the analysis of MOE 1 diluted approximately 10:1 in water. The analysis was performed on a C-8 analytical column using the developed method.
Fig. 11. Chromatogram resulting from an 80 microliter injection of MOE 1 diluted approximately 10:1 in water. The analysis was performed on a C-8 analytical column using those conditions found optimum for the C-18 analytical column (gradient C-18). The chromatogram was plotted at 250 millivolts.
Table 2. Peak areas obtained from injections of a 1.06 ug/mL Carbaryl standard prepared by diluting a 105.5 ug/mL concentrated Carbaryl solution 100:1 in water.

<table>
<thead>
<tr>
<th>Injection Volume (ul)</th>
<th>Peak Area Counts (x10^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>232</td>
</tr>
<tr>
<td>50</td>
<td>447</td>
</tr>
<tr>
<td>75</td>
<td>661</td>
</tr>
<tr>
<td>100</td>
<td>867</td>
</tr>
</tbody>
</table>

\[ y = 8.4734 \times 10^3 X + 2.2457 \times 10^4 \]
equilibrated with mobile phase. In the case of the gradient elution program for the C-8 analytical column, the initial mobile phase consisted of 30% acetonitrile/70% water. After valve switching to the sample 'load' position, distilled water was pumped through the 5 micron C-18 Brownlee pre-column at 2.5 mL/min for 10 minutes to flush all acetonitrile from the sorbent. The Brownlee columns were chosen for the initial experiments by virtue of their commercial availability and ease of replacement through a cartridge type holder. A 100 mL water sample was then loaded at 5 mL/min resulting in a system backpressure of approximately 3500 pounds per square inch (psi). After the completion of the sample loading step, the valves were switched to the sample 'elute' position and the analytes were flushed to the analytical column by the application of mobile phase through the pre-column. The observed Carbaryl peak areas from the 1.06 ppb sample on-line pre-concentration runs were on average 95.3% the area of those observed from 100 microliter injections of a 1.08 ppm concentrated standard (both representing an absolute amount of approximately 100 ng of analyte, Table 3). Pump effluent was monitored and the calculated peak areas were corrected for actual pump flow production, although failure to do this would result in only 1.5% error (105 mL actual delivery for a 20 minute sample loading step at 5 mL/min). The initial experiments with Carbaryl were apparently successful as peak areas between standard injections and sample runs are nearly quantitative (Table 3). The lack of breakthrough of the analyte is also evidenced by these data.
Table 3. Comparison of Carbaryl peak areas from 3 consecutive on-line pre-concentration sample runs. Peak area counts from 3 replicate sample runs of a 100 mL 1.06 ppb sample were compared with those from a 100 microliter injection of a 1.06 ppm standard.

<table>
<thead>
<tr>
<th>Replicate Sample Run</th>
<th>Percentage of standard peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.5</td>
</tr>
<tr>
<td>2</td>
<td>96.9</td>
</tr>
<tr>
<td>3</td>
<td>93.5</td>
</tr>
</tbody>
</table>
Preliminary On-line Pre-concentration Experiments with Carbamate Pesticides

Attention was then turned to attempting to pre-concentrate a larger number of selected pesticides. These pesticides are shown in Table 4 along with the calibration data for each compound. Fig. 12 was produced from a sample prepared by diluting the concentrated standard solution MOE4 (Appendix 5) 1000:1. Peak area data for the pesticides is shown in Table 4. Data is not presented, however, for Bux, Butylate, and Diallate as these compounds gave confusing results. As seen in Fig. 12, Bux broadens into a dish-topped peak with an area much greater than that obtained from the Bux peak observed from an injection of the concentrated standard. The literature describes the compound as a mixture. However, this does not explain the discrepancy in the peak areas as they should be quantitative. The peak area observed in the pre-concentration run is almost twice that observed in the chromatogram resulting from the standard injection. Butylate and Diallate recoveries were not reported as the two compounds could not be separated well enough to enable quantitation of either component.

Initial On-line Pre-concentration Experiments Using Municipal Tap Waters

As seen in Fig. 13, the analysis of tap water samples presents concerns that were not evident in the analysis of samples prepared in distilled water. Chromatograms of municipal tap water samples
Table 4. Peak area comparisons from 3 consecutive sample runs. Standard MOE 4 was diluted 1000:1 in distilled water and 100 mL was loaded onto the pre-column at 2.5 mL/min. The resulting peak area counts were compared with those obtained from a 100 uL injection of MOE 4.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Percentage of standard peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran</td>
<td>97.4</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>98.9</td>
</tr>
<tr>
<td>Propham</td>
<td>99.9</td>
</tr>
<tr>
<td>Bux</td>
<td>N/A</td>
</tr>
<tr>
<td>Cl-Propham</td>
<td>102.4</td>
</tr>
<tr>
<td>Barban</td>
<td>94.3</td>
</tr>
<tr>
<td>EPTC</td>
<td>104.9</td>
</tr>
<tr>
<td>Butylate</td>
<td>N/A</td>
</tr>
<tr>
<td>Diallate</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Fig. 12. Chromatogram resulting from the on-line pre-concentration of 100 mL of sample prepared by the dilution of MOE 4 1000:1 in distilled water. The sample loading rate was 2.5 mL/min. Separation was performed on a C-8 analytical column using the developed method. The chromatogram was plotted at 200 millivolts.
Fig. 13. Chromatogram resulting from the pre-concentration of 100 mL of municipal tap water using the described method. The chromatogram was plotted at 200 millivolts.
are characterized by large peaks due to early eluting material. These results are similar to those obtained by other authors from the analysis of similar sample matrices (53). There is obviously a large amount of this relatively polar material retained by the pre-column and several of these components are sensitive to UV detection. Fig. 14 shows a chromatogram resulting from a sample prepared in municipal tap water. Fortunately, most of the aforementioned impurities have eluted and the baseline is sufficiently stable at later time (greater than 15 minutes) to allow for determination and quantitation of all of the desired analytes.

Table 5 lists the pesticides and their percentage peak areas (as compared to those peak areas obtained from injections of a concentrated standard) from 5 replicate municipal tap water sample runs. The water samples were prepared by diluting MOE 7 (Appendix 6) 1000:1 in water. The peak areas are close to those obtained from standard injections of MOE 7 for the majority of the analytes, with the exception of Aminocarb and Eptam. Injections of the concentrated Aminocarb standard solution indicated that the peak profile was much sharper when the C-18 pre-column was taken off-line. This suggests that calibration injections should be made with the pre-column off-line on order to obtain the sharpest peak profile. The sharper peak profile is more like that obtained from the actual pre-concentration sample runs. Quantitation of a broader peak profile is made difficult, as the determination of the threshold at which the peak end meets the baseline is more difficult than in a case when the peaks were sharper. Ideally, the chromatography software program (in this case, the Waters 840 system) should be
Chromatogram resulting from the pre-concentration of a 100 mL sample prepared by the 1000:1 dilution of MOE 7 in water. The sample was loaded onto a 3 cm 5 micron C-18 pre-column at 2.5 mL/min and analysed by the described method with the exception that backflush elution was employed.
Table 5. Peak area comparisons from 5 replicate sample runs. Standard MOE 7 was diluted 1000:1 in a municipal tap water and loaded onto a C-18 pre-column at 5 mL/min. The resulting peak areas were compared with those from a 100 uL injection of MOE 7.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Average Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminocarb</td>
<td>64.8</td>
</tr>
<tr>
<td>Propoxur</td>
<td>91.4</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>91.8</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>92.2</td>
</tr>
<tr>
<td>Propham</td>
<td>89.5</td>
</tr>
<tr>
<td>Captan</td>
<td>79.0</td>
</tr>
<tr>
<td>Cl-Propham</td>
<td>90.5</td>
</tr>
<tr>
<td>Barban</td>
<td>97.3</td>
</tr>
<tr>
<td>EPTC</td>
<td>76.6</td>
</tr>
</tbody>
</table>
able to precisely determine the true baseline but this is seldom the case and all of the data presented here have required manual checking of the baseline positioning by the software. This may have been a factor in the smaller Aminocarb peak areas. Aminocarb is the earliest eluting of the analytes, so sample breakthrough must also be a consideration for this compound.

Summation of Preliminary Studies

Up to this point, all of the data presented were obtained from samples prepared by the dilution of concentrated combined standards. These combined standards were made up by the dilution of the individual standard stock solutions of the individual pesticides. These individual standards were diluted in water and stored at 4°C until needed. The pesticides chosen for the study are all 'stable' in aqueous solution at near neutral pH as described in the literature (see Appendix 1) but will certainly degrade slowly over time due to hydrolysis and at differing rates. For this reason, subsequent combined standards were diluted in organic solvent instead of water (with the exception of Benomyl) and were found to be much more stable over longer periods of time.

It was decided that, based upon the priorities of the Ontario Ministry of the Environment, that the pesticides used in any further studies would be limited to those listed in Table 8. These pesticides are also those listed in the experimental section. Bux, Diallylate, and Eptam were no longer expected to be of concern in Ontario environmental samples. A decision was made to develop a
separate analytical procedure for Benomyl as residues of this compound are difficult to determine. It was decided to include Aminocarb in the group of pesticides once a procedure had been developed for Benomyl.

A C-8 analytical column was deemed to be most appropriate for further studies as it is the column of choice in the current Ontario Ministry method (1). However, a suitable gradient elution program for the separation of the carbamate pesticides on a C-18 analytical column was developed.

B. ON-LINE PRE-CONCENTRATION

Unidirectional and Backflush Elution

The valving system employed in the on-line pre-concentration apparatus (Fig.15) allows for application of the mobile phase to the pre-column in the same direction in which the sample was loaded (unidirectional elution), or in a direction opposite to that in which the sample was loaded (backflush elution). This makes the system more versatile than those employing only one high pressure valve. Our studies revealed close similarities between unidirectional and backflush elutions for a C-18 pre-column (Fig. 16). This is in sharp contrast to results obtained by straight injection of a concentrated stock solution (1000 x the concentration of the standard water samples used for this study) into the HPLC (Fig.17). The peak heights and shapes of the earlier eluting analytes are very different than those from the pre-concentration sample runs. However, no
Fig. 15

Schematic of the valve-switching system. V, P, and F denote valves, pumps, and filters respectively. During the sample loading step, P1 dispenses sample. During the elution steps, P1 dispenses water and P2 acetonitrile as part of the mobile phase.

**SAMPLE LOAD**

**UNIDIRECTIONAL ELUTION**

**BACKFLUSH ELUTION**
Fig. 16. Chromatograms showing the effect of backflush elution (16A) and unidirectional elution (16B) for a 5 micron C-18 pre-column.

Each chromatogram is plotted at 90 millivolts. The samples were prepared and analysed by the method described in the experimental section.
Fig. 17. Chromatogram resulting from a 150 microliter injection of the spiking standard diluted to 30% acetonitrile in water and analysed by the developed method.
difference was found in the average peak area counts by both methods. Adsorption onto, and elution from, the pre-column increases peak sharpness for Propoxur and Carbofuran, although the relative retention times for the two compounds are shifted closer together.

**Statistical Calculations of System Performance**

Calculations reveal that the capacity factors for the early eluting components in the on-line pre-concentration experiments (Propoxur and Carbofuran) are approximately half those obtained for the same two compounds using a straight injection method (Tables 6 and 7). Theoretical plate numbers for the two compounds calculated using on-line pre-concentration are approximately four times those measured using straight injection (Tables 6 and 7). Although resolution of the two compounds is better using the straight injection ($R = 1.6$), the on-line pre-concentration method gave good separation of the two compounds ($R = 1.0$).

It appears that the pre-column is a factor in the separation of the analytes by the analytical column. Fig. 18 is a chromatogram produced by unidirectionally eluting the analytes from the C-18 pre-column directly to the UV detector without separation on an analytical column. From close scrutiny of Fig. 16, it appears that the peak shapes in the chromatogram resulting from unidirectional elution are marginally sharper than those obtained from backflush elution. This suggests that the analytes move a shorter distance on
Table 6. Table of pesticides showing retention times, capacity factors (K), and number of theoretical plates (N), for each compound. The resolution between the two earliest eluting compounds, Propoxur and Carbofuran, has also been calculated. The calculations were made using the results from an injection of the concentrated standard (MOE 10, diluted to 30% in water) directly onto the analytical column.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>R.T. (min)</th>
<th>K</th>
<th>N</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur</td>
<td>12.34</td>
<td>6.48</td>
<td>3807</td>
<td></td>
</tr>
<tr>
<td>Carbofuran</td>
<td>13.34</td>
<td>7.08</td>
<td>4450</td>
<td></td>
</tr>
<tr>
<td>Carbaryl</td>
<td>15.58</td>
<td>8.44</td>
<td>19180</td>
<td></td>
</tr>
<tr>
<td>Propham</td>
<td>18.36</td>
<td>10.13</td>
<td>41616</td>
<td></td>
</tr>
<tr>
<td>Captan</td>
<td>20.72</td>
<td>11.56</td>
<td>94226</td>
<td></td>
</tr>
<tr>
<td>Cl-Propham</td>
<td>22.03</td>
<td>12.35</td>
<td>59916</td>
<td></td>
</tr>
<tr>
<td>Barban</td>
<td>22.91</td>
<td>12.88</td>
<td>64798</td>
<td></td>
</tr>
<tr>
<td>Butylate</td>
<td>29.23</td>
<td>16.72</td>
<td>35000</td>
<td></td>
</tr>
</tbody>
</table>


Table 7. Table of pesticides showing retention times, capacity factors (K), and number of theoretical plates (N), for each compound. The resolution (R) between the two earliest eluting compounds, Propoxur and Carbofuran, has also been calculated. The calculations were made using the results of an on-line pre-concentration sample run (100 mL of MOE 10 diluted 1000:1 in water) using the developed method.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>R.T. (min)</th>
<th>K</th>
<th>N</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur</td>
<td>16.00</td>
<td>3.48</td>
<td>20227</td>
<td></td>
</tr>
<tr>
<td>Carbofuran</td>
<td>16.40</td>
<td>3.60</td>
<td>21252</td>
<td>1.0</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>17.35</td>
<td>3.86</td>
<td>30103</td>
<td></td>
</tr>
<tr>
<td>Propham</td>
<td>19.25</td>
<td>4.39</td>
<td>20333</td>
<td></td>
</tr>
<tr>
<td>Capatan</td>
<td>21.10</td>
<td>4.91</td>
<td>61621</td>
<td></td>
</tr>
<tr>
<td>Cl-Propham</td>
<td>22.25</td>
<td>5.23</td>
<td>61119</td>
<td></td>
</tr>
<tr>
<td>Barban</td>
<td>23.00</td>
<td>5.44</td>
<td>64309</td>
<td></td>
</tr>
<tr>
<td>Butylate</td>
<td>29.40</td>
<td>7.24</td>
<td>27435</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 18. Chromatogram resulting from the pre-concentration of 100 mL of sample and subsequent elution of the analytes directly to the UV detector without passing through an analytical column. The chromatogram was produced using the developed method and plotted at 150 millivolts.
the pre-column during unidirectional elution than during backflush elution.

**C-8 and C-18 Stationary Phases**

A C-8 sorbent packing in the pre-column was also investigated as an alternative to the C-18 packing. As opposed to those results obtained with the C-18 packing, backflush elution improved resolution between Propoxur and Carbofuran and all peak profiles appear sharper (Fig. 19). Since C-8 is a more polar stationary phase, the Propoxur and Carbofuran would be expected to show greater affinity for the stationary phase and will be retained nearer the head of the pre-column than the other analytes. Unidirectional elution would necessitate the Propoxur and Carbofuran having to travel most of the length of the pre-column before being carried to the analytical column. During backflush elution, the Propoxur and Carbofuran would be immediately desorbed from the pre-column and carried to the analytical column. No difference was found in the average peak area counts when using the C-18 or C-8 sorbent. In the developed method, a combination of a C-18 sorbent in the pre-column and a C-8 analytical column was employed with unidirectional elution. Backflush elution offered no advantage when using a C-18 pre-column. A C-18 analytical column was also investigated but no improvement was found in the separation of the eight pesticides.

Table 8 lists the eight selected pesticides (see also experimental section) and shows their retention times, peak area
Fig. 19. Chromatograms showing the effect of backflush (19A) and unidirectional elution (19B) for a 5 micron C-8 pre-column. Each chromatogram is plotted at 90 millivolts. The samples were analysed by the developed method.
Table 8. Summary of analytical results of eight pesticides: retention times (R.T.), average peak area counts from 5 replicate measurements using a 5 micron pre-column, sample concentrations, and minimum detectable concentrations for a 100 mL sample analysed by the developed method.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R.T.</th>
<th>PEAK AREA</th>
<th>SAMPLE CONCENTRATION</th>
<th>MINIMUM DETECTABLE CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>(x10³)</td>
<td>(ppb)</td>
<td>(ppt)</td>
</tr>
<tr>
<td>1. Propoxur</td>
<td>16.00</td>
<td>598±3</td>
<td>3.84</td>
<td>65</td>
</tr>
<tr>
<td>2. Carbofuran</td>
<td>16.40</td>
<td>597±24</td>
<td>4.35</td>
<td>70</td>
</tr>
<tr>
<td>3. Carbaryl</td>
<td>17.35</td>
<td>377±7</td>
<td>0.42</td>
<td>10</td>
</tr>
<tr>
<td>4. Propham</td>
<td>19.25</td>
<td>580±12</td>
<td>3.17</td>
<td>50</td>
</tr>
<tr>
<td>5. Captan</td>
<td>21.10</td>
<td>164±6</td>
<td>9.70</td>
<td>460</td>
</tr>
<tr>
<td>6. Cl-Propham</td>
<td>22.25</td>
<td>242±6</td>
<td>0.98</td>
<td>30</td>
</tr>
<tr>
<td>7. Barban</td>
<td>23.00</td>
<td>252±5</td>
<td>1.08</td>
<td>40</td>
</tr>
<tr>
<td>8. Butylate</td>
<td>29.40</td>
<td>428±10</td>
<td>4.07</td>
<td>150</td>
</tr>
</tbody>
</table>

The pesticides are numbered to coincide with those in the figures.
counts with the values of co-efficient of variation from 5 replicate measurements at the concentrations listed, and the minimum detectable concentrations using the developed method. The reproducibility of the method is excellent as evidenced by an average value of coefficient of variation of approximately ±2% for all of the compounds of interest. Of the eight compounds, only Captan is not of the carbamate class of pesticide. The minimum detectable concentrations were calculated based on a 100 mL sample using a 3 to 1 signal to baseline noise ratio. The detection limit can be influenced by the number and concentration of co-eluting impurities in the sample matrix. The minimum detectable quantities can vary depending upon sample volume and sample concentration. Ultimately, the key factor for the determination of the detection limit for a given sample matrix will be the number and concentration of co-eluting impurities.

**Minimum Detectable Concentrations**

Figure 20 shows a chromatogram resulting from a distilled water sample with low concentrations (one tenth of the concentrations listed in the sample concentration column of Table 10) of the eight pesticides. At these concentrations, the peaks from the impurities in the sample matrix are roughly equal in height and area to those of the sample peaks. From this chromatogram, it is evident that the method approaches its practical detection limits at these analyte concentrations. Background subtraction (as shown in Fig. 20) could be a valuable asset in the quantitation of analytes, but
Fig. 20. Chromatograms corresponding to a blank (20B), sample (20A), and background subtraction (20C) from a distilled water sample pre-concentrated on a 10 micron Ultrasil ODS pre-column.

The concentrations of the pesticides are one tenth those listed in Table 10. Each chromatogram is plotted at 90 millivolts. The samples were analysed by the developed method.
the absence of a blank for field samples makes this impossible. Baseline correction (otherwise known as gradient subtraction) could be used to improve the chromatogram profile but a solvent blank does not accurately duplicate the conditions to which the pre-column has been subjected. The valve switch after the sample loading step results in residual sample water being flushed to the analytical column. Over the course of many sample analyses, this must be of concern, as the performance of the analytical column must be expected to decline. It is impossible to duplicate this procedure to produce a solvent blank. A practical alternative may be to use 100 mL of the purest water available as a sample blank and to subtract the resulting gradient profile (this can be done by the chromatography computer software). In the case of more heavily contaminated samples, the effect of minute impurities in the 'blank' should be negligible.

**Packing Size of Sorbent in Pre-column**

Separation of the analytes when using a 40 micron sorbent is good but the peak areas for the first 3 eluting compounds (Propoxur, Carbofuran, and Carbaryl) are not as large as those of the 5 micron material (Fig. 21). The peak areas with the 10 micron Ultrasil ODS sorbent were as large as those of the 5 micron sorbent except for Propoxur and Propham whose peak areas were 65% and 75% respectively of those obtained with the 5 micron sorbent. The 5 micron material shows excellent retention of all 8 analytes. From these results, it was concluded that the use of the 5 micron sorbent
Fig. 21. Chromatograms showing the effect of sorbent particle diameter upon the retention of analytes. Chromatograms correspond to 40 micron (21A), 10 micron (21B), and 5 micron (21C) packings. Each chromatogram is plotted at 90 millivolts. The samples were analysed by the developed method.
was most appropriate as no breakthrough of the early eluting pesticides was observed.

Sample Loading Rate

The results of the investigation of sample loading rate indicated no variation of retention of analytes under practical conditions. The flow rates through the pre-columns were increased in 1 mL/min increments from 3 mL/min to 6 mL/min for the 5 micron packing (Fig. 22) and 3 mL/min to 7 mL/min for the 10 micron packing (Fig. 23). In both cases, the flow rates did not significantly affect the retention of analytes by the pre-column. Investigation of higher flow rates using the Model 501 single head pump to determine the point of sample breakthrough was not possible due to restrictions imposed by the high column backpressures at flow rates exceeding 6 mL/min for the 5 micron packing and 7 mL/min for the 10 micron packing. However, experiments with a dual head pump (Model 600 Powerline) and a 5 micron pre-column showed that sample loading rates of 10 mL/min can be attained without breakthrough of any of the analytes (Fig. 24). This result is in good agreement with results obtained by Goewie et al. (27). With the Model 501 pump, a sample loading rate of 5 mL/min with the 5 micron packing was most appropriate. Under these conditions, a total sample loading time for 100 mL is 20 min, which is quite adequate considering the subsequent 35 min chromatographic step. A completely automated procedure (including sample loading and analysis) takes approximately 60 minutes with
Fig. 22. Chromatograms showing the effect of sample loading rate upon retention of analytes for a 5 micron C-18 pre-column.

Chromatograms correspond to 3 mL/min (22A), 4 mL/min (22B), 5 mL/min (22C), and 6 mL/min (22D). Chromatograms are plotted at 90 millivolts. The samples were analysed by the developed method.
Fig. 23. Chromatograms showing the effect of sample loading rate upon retention of analytes for a 10 micron C-18 pre-column.

Chromatograms correspond to 4 mL/min (23A), 5 mL/min (23B), 6 mL/min (23C), and 7 mL/min (23D). Chromatograms are plotted at 90 millivolts. The samples were analysed by the developed method.
Fig. 24 Chromatogram resulting from an analysis in which the sample loading rate was 10 mL/min. The concentrations of the pesticides were the same as those listed in Table 10 except for Propoxur, which was present in the sample at 3.5 ppb. The sample was analysed by the developed method.
the developed method. If the concentrations of carbamate residues are at least 10 times those investigated in our study, or the detection limits achieved by conventional liquid-liquid extraction techniques are acceptable, then a sample size of only 5 or 10 mL is needed and the analysis time becomes much shorter. It is important to note that the minimum detectable concentrations with 100 mL of sample by the developed method is capable of detecting these pesticides at concentrations at least one order of magnitude less than those methods currently being used (1).

Pre-column Longevity

The longevity of the pre-column is an important economic consideration when making a choice between an SPE or an on-line pre-concentration technique. Commercially available SPE cartridges are substantially cheaper ($1.50 to $2.50 Canadian) than 5 micron pre-columns ($60.00 Canadian). However, in our study, one 5 micron pre-column stood up well to the analysis of at least thirty 100 mL distilled water samples without showing any noticeable deterioration. The amount of deterioration was assessed by monitoring the resolution between the Propoxur and Carbofuran, and by monitoring peak tailing of all analyte peaks (Fig. 25). On a cost per analysis basis, the technique can compete with a method employing commercial SPE cartridges. The pre-column could be used substantially longer if only 10 mL or less of sample is used. The cost of operation of the method then becomes much lower.
Fig. 25. Chromatogram showing the effect of prolonged use of a 5 micron C-18 cartridge. The sample was analysed by the developed method. The chromatogram was plotted at 90 millivolts.
Sample Matrix Study

The developed method was applied to the analysis of several kinds of water samples. Chromatograms of these water samples are shown in Fig. 26 through 30. Each water sample showed several peaks but most are unique to individual samples. The tap water samples are prone to huge peaks early in the chromatogram from early eluting impurities but the baseline is sufficiently stable after 15 minutes to allow for accurate quantitation of analytes. These large peaks might be eliminated from the chromatograms by allowing the mobile phase to pass through the pre-column for a set period of time while the valve apparatus is still in the sample load position. This would effectively 'vent' the early eluting impurities to waste. The valves could then be switched to the elute position for the determination of the desired components. This procedure would also prevent residual sample water in the system from reaching the analytical column and would extend the performance of the analytical column for longer periods of time.

Instrument Calibration

The method of quantitation of analytes that was originally followed was to calibrate the instrument by four injections of increasing volume of a concentrated stock solution without employing the pre-concentration procedure, and subsequently comparing the peak areas. This approach was, however, found to be unacceptable as this method of calibration necessitates the
Fig. 26. Chromatograms of distilled (26A), one of the best HPLC grade (26B), and reverse osmosis (26C) waters. Each chromatogram is plotted at 15 millivolts. The samples were analysed by the developed method.
Fig. 27. Chromatograms of two commercial bottled spring waters.

Chromatogram (27A) is a Canadian product and chromatogram (27B) corresponds to a European product.

Each chromatogram is plotted at 40 millivolts. The samples were analysed by the developed method.
Fig. 28. Chromatograms comparing two municipal tap waters. Each chromatogram is plotted at 40 millivolts. The samples were analysed by the described method.
Fig. 29. Chromatograms comparing laboratory distilled (29A), and deionized (29B) waters. Chromatograms are plotted at 40 millivolts. The samples were analysed by the developed method.
Fig. 30. Chromatograms showing a comparison of two other commercial HPLC grade waters. Chromatograms are plotted at 40 millivolts. The samples were analysed by the developed method.
comparison of data obtained by two differing experimental
techniques. Although this method of calibration may be deemed to
be invalid, the analyst may feel confident that, if peak areas
between the methods are quantitative, that the analytes are being
satisfactorily adsorbed during sample loading and desorbed during
elution. As demonstrated in Fig. 16 and 17, the peak shapes are
substantially different although by area integration all compounds
showed quantitative relationships. Because of this difference, three
concentrations of standard solutions were prepared (for the
majority of the analytes at 0.1, 1.0, and 10 ppb) and the resulting
peak areas were calculated by using the developed method. Because
the response of the compounds was linear (Appendix 3, Table 9, Fig.
31), it was concluded that the peak area counts from the pre-
concentration of the 1 ppb standard were adequate for daily
calibration.

C. SOLID PHASE EXTRACTION

Sample Loading Rate

In the development of the methodology, several experimental
parameters were investigated. A sample loading rate of 10 mL/min
through the cartridge was found to be optimum. At a sample loading
rate of 20 mL/min, breakthrough of the early eluting compounds
(Propoxur, Carbofuran, and Carbaryl) was evident. This was
confirmed by the analysis of the sample water, collected after
Table 9. Tabulation of average peak area counts from on-line pre-concentration samples over three orders of concentration.

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.1 ppb (x10^2)</th>
<th>1 ppb (x10^3)</th>
<th>10 ppb (x10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur</td>
<td>600</td>
<td>598</td>
<td>618</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>620</td>
<td>597</td>
<td>580</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>400</td>
<td>377</td>
<td>397</td>
</tr>
<tr>
<td>Propham</td>
<td>520</td>
<td>580</td>
<td>553</td>
</tr>
<tr>
<td>Captan</td>
<td>175</td>
<td>164</td>
<td>162</td>
</tr>
<tr>
<td>Cl-Propham</td>
<td>300</td>
<td>242</td>
<td>263</td>
</tr>
<tr>
<td>Barban</td>
<td>270</td>
<td>252</td>
<td>230</td>
</tr>
<tr>
<td>Butylate</td>
<td>400</td>
<td>428</td>
<td>459</td>
</tr>
</tbody>
</table>
Fig. 31. Chromatogram resulting from the analysis of a sample containing ten times the concentration of those listed in Table 10 (with the exception of Propoxur which is present at 3.3 ppb). The sample was analysed by the developed method.

\[ \times 10^{-1} \text{ volts} \]
passing a sample through the cartridge, by the previously described method (26) (Fig. 32).

**Cartridge Packing Size**

A comparison of the retention capabilities of the Sep-Pak Custom C-18 (40 micron) cartridges and the Sep-Pak Plus C-18 (90 micron) cartridges revealed no significant differences in the retention of any of the analytes (Fig. 33).

**Choice of Eluting Solvent**

Acetonitrile, chosen as an elution solvent as it eluted all of the analytes from the cartridge, is water miscible and is suitable for reversed-phase HPLC analysis. Methanol also satisfactorily eluted all the analytes, but because acetonitrile was used in the mobile phase, it was chosen as the standard elution solvent. Other solvents may have greater eluting power in reversed-phase chromatography but are not water miscible. This is an important consideration when choosing an elution solvent as non-water miscible solvents will not come into intimate contact with the stationary phase due to the presence of residual sample water being present on the sorbent. A volume of 10 mL of hexane passed through the Sep-Pak Plus as the elution solvent failed to result in satisfactory recoveries (on average, 30%). This study indicated that it was difficult to eliminate all of the residual water from a C-18 stationary phase, even when it is subjected to extended periods of
Fig. 32. Chromatogram resulting from the on-line pre-concentration analysis of the sample effluent from the Sep-Pak Plus cartridge during a sample loading step where the flow rate through the SPE cartridge was 20 mL/min. The sample was analysed by the developed on-line pre-concentration method.
Fig. 33. A comparison of the retention of analytes by (33A) 40 micron C-18 (Sep-Pak Custom cartridge) and (33B) 90 micron C-18 (Sep-Pak Plus cartridge). Chromatograms were produced by injection of 0.175 mL of eluate without a blow-down step. Chromatograms are plotted at 25 millivolts. The sample was analysed by the developed method.
air drying. This was confirmed by the presence of water in the eluate, even after a 30 minute cartridge air drying step (Appendix 2). To evaporate the eluate, a 15 minute blow-down time with a gentle stream of nitrogen (at approximately 5 psi) was chosen. Under these conditions, reproducible results (coefficients of variation of approximately 2%) were obtained; there was essentially no loss of analytes owing to splashing or co-evaporation.

**Amount of Solid Sorbent Contained in the Cartridge**

The Sep-Pak Plus and Sep-Pak Light cartridges were compared in this study to determine the weight of solid sorbent needed to retain the analytes. The results suggest that the Sep-Pak Light cartridge is not only adequate to retain all the analytes under the conditions described, but is also superior to the Sep-Pak Plus cartridge. Fig. 34 and 35 show separations using the Sep-Pak Light and Plus cartridges, respectively, in the analysis of tap water samples. The two chromatograms show similar capacity for the early eluting material present in the sample matrix. However, these early eluting peaks are much smaller than those obtained from the Brownlee 3 cm 5 micron pre-columns in the on-line pre-concentration experiments, indicating that there is breakthrough of the early eluting impurities. This was confirmed by the analysis of the sample effluent from the cartridges by the on-line pre-concentration method. This breakthrough was observed only with the early eluting impurities, and all the carbamates studied were quantitatively recovered. These findings indicate that the use of
Fig. 34. Chromatogram resulting from the analysis of a municipal tap water using the developed SPE methodology and the Sep-Pak Plus cartridge.
Fig. 35. Chromatogram resulting from the analysis of a municipal tap water using the described SPE methodology and the Sep-Pak Light cartridge.
the SPE cartridge is advantageous not only for the concentration of analytes, but also for the cleanup of specific impurities. The chromatograms produced from tap water samples using the SPE cartridges are much 'cleaner' than those produced from the on-line method. The baseline becomes stable in a relatively short period of time (7.5 min), and the analytical column is being subjected to far less early eluting material. This may become more important if the analyst wishes to employ the method in the analysis of more polar (earlier eluting) analytes.

The air drying step did not remove the majority of the residual sample water from the larger Sep-Pak Plus cartridge. Consequently, when the eluant was passed through the Sep-Pak Plus cartridge, it became sufficiently diluted with water that a fraction of the eluate could be injected directly into the HPLC without further dilution with water for analysis of the majority of the pesticides (Fig. 33). The resolution between Propoxur and Carbofuran, however, was not as good as with a straight injection of the concentrated injection standard (Fig. 17) which was prepared at 30% acetonitrile in water (the same percentage composition of the mobile phase at the starting of the gradient program). This suggested that the percentage of acetonitrile in the eluate was somewhat higher than 30% (Fig. 33). When the percentage of organic solvent in the sample is greater than that in the mobile phase, a 'solvent effect' results as evidenced by peak broadening of the analytes (74). In our case, any peak broadening will be quite noticeable as the resolution between the first two eluting components, Propoxur and Carbofuran, will decrease. This is acceptable if the poorer resolution is not of
concern and if maximum sensitivity is not necessary. If the eluate blow-down step has not been performed, only approximately 15% of the eluate from the cartridge can be injected into the HPLC. Our studies have shown that all of the residual sample water on the Sep-Pak Plus cartridge is displaced by the first 0.50 mL of acetonitrile. Injection of a 175 microliter aliquot of the first 0.5 mL of eluate from the Sep-Pak Plus cartridge showed no solvent effect but a solvent effect was apparent from the injection of an aliquot from the second 0.5 mL of eluate.

Study of Residual Water on the Cartridge

The residual water on the Sep-Pak Plus cartridge is a disadvantage because it lowers the volatility of the eluted solvent under the nitrogen stream. Forcing the evaporation of this eluate resulted in the loss of analytes, particularly Butylate. In contrast with the Sep-Pak Plus cartridge, the air drying step eliminated most of the residual sample water from the Sep-Pak Light cartridge. This may be due to the fact that the flow of air through the Sep-Pak Light is more concentrated than that through the Sep-Pak Plus, as the sorbent is restricted to a narrow tube as opposed to a large bulb. Direct injection of the eluate from the Sep-Pak Light resulted in a significant solvent effect as the percentage of organic solvent (acetonitrile) in the sample was much greater than that of the mobile phase (Fig. 36). This solvent effect was still apparent even if the air drying step was eliminated and the residual sample water was collected as part of the eluate. We have estimated that
Fig. 36. Chromatogram resulting from the injection of a 175 microliter aliquot of the eluate from the Sep-Pak Light cartridge before the evaporation step under nitrogen. Otherwise, the developed method is used.
approximately 0.10 mL of water remained on the sorbent after a brief 15 second air drying step. To aid in this estimation, injections of the concentrated standard diluted to varying percentages of organic solvent were made in order to observe the degree of solvent effect associated with increases in organic solvent percentage (Figs. 37-40). An example of the estimation of the residual water remaining on the Sep-Pak Light cartridge can be explained as follows. A 0.750 mL volume of eluate from a Sep-Pak Light (3 x 0.250 mL of acetonitrile) was blown down under nitrogen to 0.25 mL. Water (0.250 mL) was added to the sample to make up the final sample volume to 0.500 mL. Of this sample volume, 0.175 mL was injected. No solvent effect was observed. If the eluate from the Sep-Pak Light had been 100% acetonitrile (as might have been expected after blowing air through the cartridge to eliminate residual water), the injection would have resulted in a significant solvent effect as the percentage of organic solvent making up the sample should have been 50%. Since no solvent effect was observed, it can be concluded that the percentage of organic solvent in the sample was approximately 30% at most, or 0.150 mL. Subtracting 0.150 mL of acetonitrile from the 0.250 mL of the original sample volume before the addition of water, results in a volume of 0.100 mL that must be water. A sample volume of at least 0.350 mL is needed and if 30% (0.90 mL) of this is acetonitrile and if the eluate contains at least 0.100 mL of water, then the eluate can be blown down to 0.200 mL (from 0.750 mL) and made up to 0.350 mL with water. This results in a sample suitable for injection into the HPLC. An overall estimation of the residual sample water remaining on the
Fig. 37. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 20% acetonitrile in water using the developed method.
Fig. 38. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 30% acetonitrile in water using the developed method.
Fig. 39. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 40% acetonitrile in water using the developed method.
Fig. 40. Chromatogram resulting from a 175 microliter injection of the concentrated standard diluted to 50% acetonitrile in water using the developed method.
cartridge after the air drying step was made by varying the volume of the eluate after the nitrogen blow-down step and observing the peak profiles of the analytes.

A longer period of air drying may have eliminated more of the residual water, but this was not necessary. A gentle 15 minute blow-down of the eluate with nitrogen at 5 psi results in a 0.20 mL ± 0.01 mL sample that can be diluted to 0.35 mL with water (resulting in a sample composition of not greater than 30% acetonitrile) of which 0.175 mL can be injected. A sample solution of at least 0.30 mL is needed to adequately flush the sample injector and sample loop assembly with sample to attain a reproducible injection. Since the Millilab probe tip cannot aspirate 100% of the liquid in a container, the prepared sample volume is 0.350 mL. Since 0.175 mL of this can be injected, 50% of the original sample can be analysed. A greater sample volume might be injected if a larger sample loop were installed, but this would require a greater sample volume in order to flush the injector assembly and sample loop.

The methodology employing the Sep-Pak Light cartridges is judged to be superior as the analytes can be retained and collected with one half the volume of eluant needed with the Sep-Pak Plus cartridges (0.750 mL vs. 1.50 mL respectively). Fig. 41 shows the relative amounts of analytes removed from the Sep-Pak Light cartridge in 0.25 mL increments. The solvent eluated from the Sep-Pak Light is sufficiently volatile for evaporation under nitrogen. It should be stressed that the flow of nitrogen onto the eluate in the evaporation step must be gentle to avoid losses of analytes. Originally, attempts were made to blow-down the eluate from the
Fig. 41. Chromatograms showing the relative amounts of the analytes removed from the Sep-Pak Light cartridge by successive 250 microliter acetonitrile eluant volumes. Chromatogram (41A) corresponds to the first 250 microliters and chromatogram (41C) to the last 250 microliters. The samples were analysed by the developed method.
Sep-Pak Light to a volume of approximately 0.10 mL. An addition of 0.250 mL of water made up the solution to the required volume of 0.35 mL (resulting in a sample composition of less than 30% acetonitrile if the original eluate is 100% organic solvent). This procedure was found to be unsatisfactory as Butylate recoveries averaged only 40% (Fig. 42). Throughout this study, it was known that it was possible to recover a satisfactory percentage of the analytes (including Butylate) as a blow-down under nitrogen of 0.750 mL of the combined standard to 0.100 mL, and subsequent injection of 0.175 mL after dilution of the sample to 0.350 mL in water, showed no evaporative losses of Butylate. An attempt was made to keep the Butylate in solution by the use of a 'holding agent' (78). Five to ten percent solutions of ethylene glycol in methanol (as the two are more miscible than ethylene glycol/acetonitrile) were studied as eluting solvents. It was hoped that ethylene glycol, being non-volatile, could keep the Betylate in solution through hydrogen-bonding to prevent co-evaporation. The low volatility of the holding agent made it too difficult to blow-down the eluate to the 0.100 mL volume desired. Obtaining the ethylene glycol in high enough purity was also a problem.

The low Butylate recoveries led to the conclusion that the amount of water remaining on the cartridge after the air drying procedure was substantially greater than had been originally estimated. Since the approximately 0.10 mL of water remaining on the cartridge becomes part of the eluate, any attempt to blow-down the eluate to 0.10 mL necessitates the evaporation of all of the acetonitrile. It took an extended period of time to reduce the volume
Fig. 42. Chromatogram resulting from the analysis of a sample where the eluant was blown-down under nitrogen to a volume of 100 microliters. The sample was analysed by the developed method.
of eluate from 0.15 mL to 0.10 mL and during this period a large percentage of Butylate was lost (Table 10).

In the described method, the volume of the blown-down eluate is set at 0.20 mL ± 0.01 mL and then made up to a final volume of 0.35 mL with water. With this procedure, the percentage of acetonitrile in the sample solution remained lower than 30% and there was no sign of peak shape deterioration as shown in Fig. 43. Fig. 43 is a chromatogram of the eight pesticides in the combined standard prepared in the purest water found among those analysed. All pesticides are completely resolved and the peak shapes are very good.

Gradient correction can be used to improve chromatographic profile. This was not possible with the on-line pre-concentration method. Background subtraction is impractical for the analysis of real samples because of the absence of an appropriate blank. Figure 44 shows good reproducibility of the chromatograms resulting from three consecutive sample analyses by using the described method. With the described method, the recoveries of the pesticides from five replicate samples ranged from 84% to 93% (Table 11). In addition to recoveries, Table 13 includes standard deviations, prepared sample concentrations, and minimum detectable concentrations. The minimum detectable concentrations were derived from a 3 to 1 signal to baseline noise calculation. In fact, the minimum detectable concentrations are twice those of the on-line method as 50% of the sample can be injected in the SPE method versus 100% for the on-line method. The instrument was calibrated by 5 injections of incremental volumes (25, 50, 75, 100, 125...
Table 10. Average recovery of Butylate in relation to the final volume of the eluate after the blow-down step under nitrogen (average of five replicate measurements). The samples were analysed by the developed method.

<table>
<thead>
<tr>
<th>Final Volume of Eluate (mL)</th>
<th>Recovery of Butylate (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>80</td>
</tr>
<tr>
<td>0.20</td>
<td>82</td>
</tr>
<tr>
<td>0.15</td>
<td>79</td>
</tr>
<tr>
<td>0.10</td>
<td>30</td>
</tr>
</tbody>
</table>
Fig. 43. Chromatogram of a combined standard solution sample prepared in the best commercially available HPLC grade water. The analysis was done by the developed method.
Fig. 44. Chromatograms resulting from three successive injections of the combined standard sample solution by following the developed method. Chromatograms were plotted at 50 millivolts and gradient correction was made.
Table 1: Summary of analytical results of eight pesticides: retention times (R.T.), average recoveries from 5 replicate measurements, sample concentrations, and minimum detectable concentrations for a 100 ml sample analysed by the developed method.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R.T.</th>
<th>RECOVERY</th>
<th>SAMPLE CONCENTRATION</th>
<th>MINIMUM DETECTABLE CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>%</td>
<td>ug/L</td>
<td>( \times 10^{-3} ) ug/L</td>
</tr>
<tr>
<td>1. Propoxur</td>
<td>10.80</td>
<td>92±0.3</td>
<td>3.84</td>
<td>130</td>
</tr>
<tr>
<td>2. Carbofuran</td>
<td>11.40</td>
<td>91±0.3</td>
<td>4.35</td>
<td>140</td>
</tr>
<tr>
<td>3. Carbaryl</td>
<td>12.80</td>
<td>93±1.0</td>
<td>0.42</td>
<td>20</td>
</tr>
<tr>
<td>4. Propham</td>
<td>15.20</td>
<td>92±3.6</td>
<td>3.17</td>
<td>100</td>
</tr>
<tr>
<td>5. Captan</td>
<td>17.40</td>
<td>88±3.6</td>
<td>9.70</td>
<td>920</td>
</tr>
<tr>
<td>6. Cl-Propham</td>
<td>18.70</td>
<td>89±4.9</td>
<td>0.98</td>
<td>60</td>
</tr>
<tr>
<td>7. Barban</td>
<td>19.40</td>
<td>89±1.7</td>
<td>1.08</td>
<td>80</td>
</tr>
<tr>
<td>8. Butylate</td>
<td>24.90</td>
<td>84±1.2</td>
<td>4.07</td>
<td>300</td>
</tr>
</tbody>
</table>

The pesticides are numbered to coincide with those in the figures.
microliters) of the concentrated combined standard diluted to 30% acetonitrile in water (Appendix 3).

Sample Matrix Study

Ultimately, the choice of the weight of sorbent in the cartridge will depend upon the desired application. Our studies of municipal tap waters have shown that there is breakthrough of the early eluting impurities present in the sample matrix. This was confirmed by the analysis of the sample water collected after passing a sample through the cartridge by the method previously described (26). If the analyst wishes to retain these impurities as analytes, then a cartridge containing the maximum weight of sorbent should be used.

Fig. 45 shows a chromatogram resulting from the analysis of an untreated lake water. These results indicate a great potential to apply the described method to the analysis of not only drinking water, but also other types of water samples.

D. BENOMYL

Fig. 46 shows a chromatogram resulting from the analysis of a distilled water sample containing the eleven pesticides of concern in the study using unidirectional elution and a C-18 stationary phase in both the pre-column and the analytical column. Table 12 lists the pesticides, their retention times, sample concentrations, and minimum detectable concentrations. The minimum detectable
Fig. 45. Chromatogram resulting from the analysis of an untreated lake water. The sample was analysed by the developed method. This can be compared with Fig. 36 showing the analysis of a municipal tap water.
Fig. 46. Chromatogram showing the eleven pesticides used in the Benomyl, MBC study. The sample was analysed by the developed method.

The concentrations of the pesticides are the same as those listed in Table 14.
Table 12  Analytical results from the analysis of eleven pesticides: retention times (R.T.), sample concentrations, and minimum detectable concentrations for a 100 ml sample analysed by the developed method.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>R.T.</th>
<th>SAMPLE CONCENTRATION</th>
<th>MINIMUM DETECTABLE CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>( \times 10^{-9} ) g/mL</td>
<td>( \times 10^{-12} ) g/mL</td>
</tr>
<tr>
<td>1. MBC</td>
<td>6.30</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>2. Aminocarb</td>
<td>9.77</td>
<td>4.0</td>
<td>65</td>
</tr>
<tr>
<td>3. Propoxur</td>
<td>10.33</td>
<td>4.0</td>
<td>65</td>
</tr>
<tr>
<td>4. Carbofuran</td>
<td>10.77</td>
<td>4.5</td>
<td>70</td>
</tr>
<tr>
<td>5. Carbaryl</td>
<td>12.10</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>6. Propham</td>
<td>13.80</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>7. Captan</td>
<td>15.42</td>
<td>20.0</td>
<td>460</td>
</tr>
<tr>
<td>8. Cl-Propham</td>
<td>16.55</td>
<td>2.0</td>
<td>30</td>
</tr>
<tr>
<td>9. Barban</td>
<td>16.92</td>
<td>3.0</td>
<td>40</td>
</tr>
<tr>
<td>10. Benomyl</td>
<td>18.30</td>
<td>8.0</td>
<td>500</td>
</tr>
<tr>
<td>11. Butylate</td>
<td>21.33</td>
<td>5.0</td>
<td>150</td>
</tr>
</tbody>
</table>

The pesticides are numbered to coincide with those in the figures.
concentrations (MDC's) were calculated using a 3 to 1 signal to noise ratio with the exception of Benomyl and MBC. The MDC's for Benomyl and MBC were calculated using a 6 to 1 signal to noise ratio. This was done to ensure the accuracy of these values because the peak profiles of these two compounds were substantially broader than those of the other analytes. Fig. 47 is a chromatogram resulting from the analysis of a distilled water sample containing 1.3 ppb of Benomyl.

Inclusion of Buffered Mobile Phase

A buffered mobile phase is neccessary for the gradient elution program. Otherwise, the peak profile of MBC is unacceptably broad for quantitation. Even with the buffered mobile phase, peak profiles for Benomyl and MBC were much broader than the other compounds. These peak profiles were not improved even when other experimental conditions were investigated.

Study of Experimental Conditions

Experiments with a more polar pre-column stationary phase (C-8), backflush and unidirectional elution, and a different analytical column stationary phase (C-8), failed to improve peak profiles for MBC and Benomyl. The use of a C-8 analytical column improves separation of the three earlier eluting pesticides (Aminocarb, Propoxur, and Carbofuran) but the peak profile for Benomyl is poor and co-elutes with Butylate (Fig. 48). The developed method
Fig. 47. Chromatogram resulting from the analysis of a sample containing 1.3 ppb Benomyl. The sample was analysed by the developed method.

MBC is present in the sample as the degradation product of Benomyl.
Fig. 48. Chromatogram resulting from the analysis of a sample by the developed method with the exception that a C-8 analytical column was used.
includes the use of unidirectional elution from a C-18 pre-column onto a C-18 analytical column which was found to be the best combination of experimental conditions.

As shown in Fig. 46, peak widths of MBC and Benomyl are substantially broader than those of the other compounds when using unidirectional elution. As concentrations and volumes of water sample (and accordingly, sample loading time) are increased, peak widths increased. However, peak widths of MBC and Benomyl are substantially better if straight injections of concentrated Benomyl standards are made directly onto the analytical column without passing through the pre-column (Fig. 49). It was concluded that the band broadening apparent in the pre-concentration chromatograms is a result of the sample loading step.

Analysis of Benomyl as Both Solute and in the Solid State

Results obtained with backflush elution were substantially different from those obtained with unidirectional elution. As shown in Fig. 50, four peaks were observed with a sample that contained only MBC and Benomyl. It is clear that both the MBC and the Benomyl displayed two peaks each. Of the two peaks representing Benomyl, the first peak represents Benomyl which is adsorbed on particulate matter and retained on the inlet side of the pre-column (either on the 0.50 micron filter (F1) or at the head of the pre-column in Fig. 15). The second peak represents Benomyl which is present as solute in water and adsorbed on the pre-column. This presents the possibility of a method for the quantitation of Benomyl and MBC both
Fig. 49. Chromatogram resulting from a straight injection onto the analytical column of a sample containing only Benomyl and MBC. The chromatogram was plotted at 75 millivolts. The sample was analysed by the developed method.
as solute and in the solid state in water samples. It is interesting to note that the peak shape of the first Benomyl peak is substantially sharper than the second. Similar results were observed with MBC.

In the above example, Benomyl was analysed at 0.136 ppm (Fig. 50). MBC was present in the sample as the degradation product of Benomyl. As the solubilities of MBC and benomyl are approximately 3 ppm in water (65), the concentration of Benomyl was well within the solubilities of the analytes in water, but the sample analysis still yielded four peaks indicating that the solid state of Benomyl could be present. This is because the sample was prepared by a 100:1 dilution of a 13.6 ppm stock solution of Benomyl and analysed immediately. This indicates that a period of time is needed for the Benomyl to fully dissolve in the water matrix. This was evidenced by a substantial decrease in the peak area of the first peak and an increase in the peak area of the second peak when the same sample was analysed 45 minutes later (Fig. 51). The sum of the peak areas of the first and second Benomyl peaks were quantitative for both samples. Unidirectional elution yielded a large and broader single peak with a peak area equal to that of the sum of the two peaks obtained from backflush elution.

These findings reveal that consideration must be given to the basic handling procedures of water samples regarding the need for filtration. Depending on the pore size of the filters, regardless of whether they are used on-line or not, analytical results may be substantially different. This is important when analysing samples for compounds which have very low solubilities in water. Benomyl
Fig. 50. Chromatogram resulting from the analysis of a 0.136 ppm Benomyl suspension. A 10 mL sample was analysed by the developed method except that backflush elution was employed.
Fig. 51. Chromatogram resulting from the analysis, by the developed method, of the same 0.136 ppm Benomyl suspension described in Fig. 51, with the exception that the sample was analysed 45 minutes later.
is one of the compounds which merits this consideration as it is used at the 250-1000 ppm range in agriculture.

**Optimum Conditions for the Analysis of Benomyl and MBC Only**

The peak profiles of MBC and Benomyl can be much improved by elution with a higher percentage of organic solvent in the mobile phase, but this results in poor resolution of the other analytes. If Benomyl and MBC are the only compounds to be analysed, both compounds can be eluted as sharp peaks by using a gradient program that increases the percentage of organic solvent in the mobile phase in a shorter period of time (Fig. 52). Separation of the two components of Benomyl (as solute and solid state) was found to improve with increasing buffer strength of in the mobile phase.
Fig. 52. Chromatogram resulting from the analysis of a sample containing Benomyl and MBC only. The gradient used is that developed for the C-18 analytical column and is described in the on-line pre-concentration experimental section. The use of this gradient results in a faster increase in the percentage of acetonitrile in the mobile phase and substantially sharper MBC and Benomyl peak profiles.
SUMMARY AND CONCLUSIONS

A. ON-LINE PRE-CONCENTRATION

The development of the on-line pre-concentration method resulted in a qualitative and quantitative HPLC analysis procedure. If a solvent delivery system with three solvent capability (for example the Waters 600E Multisolvent Delivery System) is incorporated in the system which we used, the method lends itself easily to complete automation for a single analysis. This is achieved by computer software control which enables a switching of the valving system from the sample load position to the elute position without manual manipulation. By the addition of a simple rotary switching device, the method could be automated for analysis of many samples.

The lowest detectable concentrations described in the text could be improved upon by increasing the sample size but this would also increase the time of analysis. Analysis time, sample loading rate, and detection limit are all dependent upon each other and must be selected according to the purpose of the analysis. The technique developed in this study is applicable to all sample matrices investigated. Municipal tap water samples displayed large peaks in the early retention time regions of the chromatograms. These peaks do not interfere with analyte determination and could be avoided completely by modification of the existing method.
The study has shown that there are many factors that must be considered in the development of the most efficient on-line pre-concentration method i.e. the choice of the pre-column which allows the highest possible flow rate with the satisfactory retention of analytes. These factors, which are all directly or indirectly dependent upon each other, include; sample volume, sample loading rate, sorbent particle diameter, sorbent particle pore size, pre-column dimensions, and chemistry of the analytes and stationary phase. These factors all contribute to the lowering of detection limits and minimizing of analysis time. It is difficult to investigate all the factors thoroughly because of many restrictions, especially that imposed by the use of commercially available pre-columns. Most of the commercially available pre-columns are of considerable length and small internal diameter. As discussed by Goewie et al (27), a pre-column should be as short as possible with a large internal diameter. The effects of particle pore size is a phenomenon that is not discussed in great detail in the literature. Larger particle sizes with larger or smaller pores (resulting in better retention of certain classes of analytes), allow for higher sample loading rates and thus greater sample volumes and lower detection limits. Many commercially available stationary phases that are deemed to be 'equivalent' to each other may show to have very different retention or elution characteristics. This may be due to the efficiency of the endcapping process and whether or not any attempt has been made to 'deactivate' the remaining non-bonded silica substrate by endcapping it with smaller, less sterically hindered groupings.
Our results show that the method can compete on a cost per analysis basis with an SPE method employing commercially available cartridges, especially if lesser sample volumes are used for analysis. The method is rapid, accurate, and reproducible as evidenced by the low coefficients of variation. It is also quite sensitive as 100% of the sample can be analysed. The method is also attractive because of its simplicity. Little capital expenditure is required to bring the method on-line.

B. SOLID PHASE EXTRACTION

As demonstrated, the totally automated SPE-HPLC system developed is accurate, efficient, economical, and sensitive as 50% of the analytes present in the sample can be injected into the HPLC. The method can be employed in the unattended analysis of 30 or more samples. The method has demonstrated applicability to untreated surface waters as well as drinking waters. The Millilab is much less prone to clogging than is the on-line pre-concentration apparatus. The Millilab can currently analyse up to thirty 100 mL samples consecutively and unattended. Residual impurities remaining on the solid sorbent are not of concern as a new sorbent cartridge is used for each successive analysis.

The described method will result in the quantitation of analytes both as solute and as particulate matter. If it is desired to quantitate both fractions separately, all that is required is to modify the procedure by pre-filtering the sample water before passing through the Sep-Pak cartridge. Analytes present as
particulate matter trapped on the filter can be eluted using an organic solvent, injected into the HPLC, and determined. The analytes present as solute on the Sep-Pak can then be determined using the described method.

The instrument can also be programmed to add an internal standard at any time during the sample preparation procedure. Internal standards were considered but not used in this study, because of the large number of varying sample matrices that were analysed. It was impossible to select an internal standard suitable for all of these sample matrices.

C. ANALYSIS OF BENOMYL

The on-line pre-concentration method can be adapted for the simultaneous determination of MBC, Benomyl, and the rest of the carbamate pesticides. Also, this method can be used for the determination of MBC and Benomyl only. This technique offers advantages over those previously developed. These include the ability to determine both Benomyl and its metabolic derivative, MBC, quantitatively. Benomyl and MBC can be determined in both the solid states and as solutes. As on-line pre-concentration is the method used for determination, the technique allows quantitation of 100% of the analytes present in any given sample size. This allows the determination of Benomyl and MBC at low ppb levels. The Benomyl present in the sample is not exposed to any organic solvent until it is eluted from the pre-column. This minimizes the chance of any decomposition of the compound before quantitation.
APPENDIX 1

The following is a listing of most of the carbamate pesticides used in the study and includes alternative names, history, solubility in water, acute oral LD$_{50}$ values for rats, and methods of analysis (1).

1. **Carbaryl** (1-naphthyl methylcarbamate)
   Alternative names: Sevin
   History and properties: Introduced in 1956 by Union Carbide. A white crystalline solid. Melting point (mp) $26^\circ$C. A contact insecticide against pests of fruits, vegetables, cotton, and other cash crops.
   Solubility in water: 50 ppm at $20^\circ$C.
   LD 50: 560 mg/kg
   Residue analysis: GLC (2)

2. **Carbofuran** (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate)
   Alternative names: Furadan, Curaterr.
   History: Discovered by Niagra Chemical Division of FMC. White crystalline solid, mp 150-152$^\circ$C. A systemic and contact insecticide and nematocide.
   Solubility in water: 700 ppm at $25^\circ$C.
   LD 50: 10 mg/kg.
   Residue analysis: GLC (3), Spectrophotometry (4).

3. **Propoxur** (2-(1-methylethoxy)phenyl methylcarbamate)
   Alternative names: Aracarb, Suncide, Under, Aprocarb.
History: Introduced by Bayer in 1959. White, crystalline powder. mp 91.5°C, insecticide for pests such as cockroaches, flies, and mosquitoes.

Solubility in water: 200g/L at 20°C.

LD 50: 100 mg/kg.

Residue analysis: GC (5,6)

4. Aminocarb (4-(dimethylamino)-3-methyl phenyl carbamate)
Alternative names: Matacil

History: Introduced in 1963 by Bayer. White crystalline solid, mp 93-94°C, a non-systemic insecticide used against lepidopterous larvae.

Solubility in water: slight

LD 50: 50 mg/kg.

Residue analysis: GC (7)

5. Butylate (S-ethyl diisobutyl thiocarbamate)
Alternative names: Sutan.

History: Introduced by Stauffer Chemical in 1960. Liquid with boiling point (bp) of 138 C at 21 mm Hg. Selective herbicide well tolerated by corn against annual grass weed species.

Solubility in water: 45 ppm at 20°C.

LD 50: 4640 mg/kg (albino rabbits).

Residue analysis: GLC (1).

6. Diallate (S-2,3-dichloroallyl diisopropylthiocarbamate)
Alternative names: DATC, Avadex.

History: Manufactured by Monsanto Chemical. An amber coloured liquid with bp of 150°C at 9 mm Hg. A pre-emergence herbicide for cereal grains.
Solubility in water: 14 ppm at 25°C.
LD 50: 395-510 mg/kg.
Residue analysis: IR adsorption (1)

7. Eptam (S-ethyl dipropylthiocarbamate)
Alternative names: EPTC
History: Introduced in 1954 by Stauffer Chemical. Clear liquid with bp of 137-138°C at 30 mm Hg, aromatic odour, a selective herbicide and is useful preplant, pre-emergence, and post-emergence. Effective against annual and seedling grass species.
Solubility in water: 375 ppm at 20°C.
LD 50: 1631 mg/kg.
Residue analysis: GLC (8).

8. Barban (4-chloro-2-butynyl m-chlorocarbanilate)
Alternative names: Carbyne
History: Introduced in 1958 by Spencer Chemical. Crystalline solid. mp 75-76°C. Herbicide for control of wild oats in grains.
Solubility in water: practically insoluble.
LD 50: 2745 mg/kg.
Residue analysis: Absorbance at 550 nm (9).

9. Chlorpropham (isopropyl N-(3-chlorophenyl) carbamate)
Alternative names: CIPC
History: Introduced in 1951. Liquid at room temp., selective herbicide used for inhibition of sprouting potato tubers.
Solubility in water: 108 ppm at 20°C.
LD 50: 3800 mg/kg.
Residue analysis: Hydrolysis by sulphuric acid followed by colourimetry (10).

10. Captan (N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide)
    History: Introduced in 1949 by Standard Oil Development Co.
    White crystals (pure form), mp 175°C. Powerful protectant fungicide for foliage applications.
    LD 50: 9000 mg/kg.
    Residue analysis: Reaction with resorcinol followed by light transmission at 450 nm (11).

11. Propham (isopropyl phenyl carbamate)
    Alternative names: IPC
    History: White crystals, mp 87°C. Selective grass killer.
    Solubility in water: 250 ppm at 20°C.
    LD 50: 5000 mg/kg.
    Residue analysis: Acid hydrolysis followed by titrimetry (12).

12. Benomyl (Methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate)
    Alternative names: Benlate
    History: Developed by Dupont and introduced in 1968.
    Solubility in water: low
    LD 50: 10,000 mg/kg.
    Residue analysis: UV absorption (13).

REFERENCES


Appendix 2.

THE DEVELOPMENT OF A SOLID PHASE EXTRACTION METHOD FOR THE DETERMINATION OF CHLOROPHENOLS AND PHENOXY-ACIDS IN WATER.

INTRODUCTION

The development of a solid phase extraction (SPE) method for the determination of chlorophenols (CP's) and phenoxy-acids (PA's) in water is necessary in lieu of the poor recoveries obtained by conventional liquid-liquid extraction methods. This study utilizes much of the development work done by Paracel Laboratories Ltd. of Ottawa, Ontario, an independent laboratory.

A procedure has been described for the analysis of CP's and PA's in water using SPE as a pre-concentration and clean-up step. This method eliminates the need for tedious and time consuming liquid-liquid extractions that require large volumes of solvent (primarily dichloromethane).

Initial evaluation of the Paracel method was performed by monitoring the recoveries from spiked distilled water samples. Erratic and often high (greater than 100%) recoveries necessitated modification of the existing method. This report includes an investigation of the effect of varying elution solvent composition on analyte recovery. Also brought forward are several questions regarding the interactions involved between the sorbent, analyte, and mobile phase during the elution step.
PROCEDURE

The SPE cartridges used were 3 mL. C-18 cartridges from Baker. A point form description of the Paracel Laboratories method is as follows:

1. The SPE cartridges are conditioned by 1 mL of methanol followed by 1 mL of distilled water. The cartridge was not allowed to go dry between applications of solvent.

2. Apply 100 mL of spiked distilled water brought to a pH of 1.2 with concentrated phosphoric acid. The sample rate through the cartridge should be between 3 and 10 mL per minute.

3. Air dry the cartridge under vacuum for 10 minutes.

4. Elute the analyte using 600 microliters (uL) of ethyl acetate containing 1% tetrahydrofuran (THF) under "slight" vacuum.

5. Add 2 mL of ethereal diazomethane to the eluate. Add more diazomethane should the yellow colour not persist for more than 5 minutes.

6. Add 2 mL of iso-octane. Blow down the solution under nitrogen to approximately 1 mL.

7. Analyse by gas chromatography (GC). Area counts representing 1 mL of eluate or 100 mL of sample is calculated by multiplying the observed area counts by the recorded volume in the sample vial.

The following changes in this procedure are recommended.

1. Condition the cartridges by passing through 1 sorbent bed volume of methanol followed by 1 bed volume of ethyl acetate/THF (99 + 1%), 1 bed volume of distilled water. Do not allow the cartridge to go dry between solvent applications.

2. After the sample application step, elute the analyte with 2 X 0.5 mL portions of 50% ethyl acetate (99 + 1% THF)/50% hexane.

3. 0.5 mL of ethereal diazomethane is used in the methylation step.
4. Water can be removed from the eluant by micro-syringe. The eluant can be further dried over anhydrous sodium sulphate before transfer to the autosampler vials. The volumes transferred to the autosampler vials are recorded.

5. The sample is applied to the cartridge at a flow rate of approximately 5 mL/min. This corresponds to a vacuum pressure of approximately 10-12 mm. Hg when using the Supelco vacuum box.

6. The air drying step is omitted.

RESULTS AND DISCUSSION

In viewing the results from the series of three replicate extractions using the Paracel method (Table 1) with ethyl acetate as an elution solvent, it is apparent that the recoveries of some of the analytes, notably 2,3,4-trichlorophenol, Dicamba, 2,4-DP, Silvex, and 2,4-DB are unacceptably high.

An attempt was made to improve upon these large recoveries by adjustment of the elution solvent. A listing of all the solvent compositions investigated appears in Table 1. From these results, it appears that 50% ethyl acetate/50% hexane offers the best possibility for obtaining satisfactory and reproducible recoveries.

As well as varying elution solvent composition, several other modifications were indoctrinated into the method as the study progressed. Despite a fifteen minute drying period (as suggested by Paracel), or a 250 uL wash of the cartridge with hexane prior to the elution step (as suggested by the cartridge manufacturer), several drops of water were still apparent in the eluate. Drying the sample over anhydrous sodium sulphate prior to sample transfer to the GC autosampler vials eliminated
the water but brings into question the value of the time intensive air drying step or hexane wash.

If the drying step is eliminated completely, the resulting layer of water can be easily removed from the eluant by use of a microsyringe or pipet. If traces of water are still apparent in the chromatogram, the sample can be further dried over sodium sulphate.

The question of the source of the apparent interferences resulting in high recoveries was also addressed. The obvious choices are the cartridge itself (2), the water making up the bulk of the sample matrix, the elution solvent, and the diazomethane utilized in the methylation procedure. The results of a brief study concerning interferences is summarized in Table 2.

From Table 2, it is apparent that the chromatograms of elution solvent blanks are free of any potential interferences. These samples were prepared by adding 2 mL of iso-octane to 1 mL of solvent. The resulting solution was then blown down to approximately 1 mL to simulate an actual sample composition. The elution solvents investigated, 40% acetone/60% hexane and 30% propan-2-ol/70% hexane were being employed at the time in the study of the effect of elution solvent composition on recovery. It might be noted that apart from a large solvent peak, the acetone chromatogram is surprisingly clean.

The diazomethane was checked for interferences by making up a solution of 0.5 mL diazomethane, 2 mL of iso-octane, and 1 mL of elution solvent, in this case 30% propan-2-ol/70% hexane. The solution was blown down to approximately 1 mL under nitrogen. The results indicate that the quality of the diazomethane can significantly alter recoveries of several compounds.
The possibility of interferences coming directly from the cartridge was investigated by passing 100 mL of distilled water through the cartridge as sample. The elution solvent was 40% acetone/60% hexane. The second lot of cartridges appears to be marginally "cleaner" than the first lot although high readings are apparent for 2,4-DP and 2,4,5-trichlorophenol. This introduces the possibility of variation in recoveries from cartridge lot to cartridge lot.

The fact that 50% ethyl acetate/50% hexane appears to be a more appropriate elution solvent than 100% ethyl acetate raises interesting questions about the chromatographic properties of the analyte, solvent, and stationary phase. In "classical" reversed phase chromatography, the 50/50 ethyl acetate-hexane solvent should act as a stronger elution solvent than the 100% ethyl acetate. Are we dealing with a case of normal phase chromatographic interactions or is water miscibility the dominating factor?

Both the CP's and PA's contain polar and non-polar components in their structures. Interactions could take place between the non-polar component of the analyte and the elution solvent once the compounds have been adsorbed onto the stationary phase by non-polar Van der Waals interactions. The fact that hexane is totally ineffective in displacing water from the sorbent (as was found in the course of the study) suggests that any elution solvent that is predominantly hexane will not come into intimate contact with the stationary phase. A strong hydrophilic barrier is imposed once water is present in close proximity to the stationary phase. The original hydrophobic barrier imposed by the C-18 stationary phase was overcome in the conditioning step by "wetting" the packing with methanol. From Table 1, it can be seen that an elution solvent of 2%
methanol/98% hexane is quite ineffective in recovering analyte from the cartridge.

Conversely, a wash step employing 0.5 mL methanol prior to elution with 50% ethyl acetate/50% hexane resulted in an average of 25% recovery of analyte indicating that approximately 75% of the analyte was removed from the cartridge during the methanol wash step. This suggests that methanol is a strong elution solvent but methanol is also completely miscible in water.

Further study should be directed towards the question of whether water miscibility or normal phase interaction is the key factor in recovery of the analyte. Ethyl acetate is the most non-polar of the organic solvents other than THF that is sparingly water soluble. An investigation of methanol, THF, and ethyl acetate should be carried out as these three solvents cover a wide range of polarity and are water miscible to the extent required in this type of study. The results of such an investigation would help to choose appropriate elution and conditioning solvents for use in reversed phase SPE where large volumes of water comprise the bulk of the sample matrix.

A larger number of replicate extractions using 50% ethyl acetate/50% hexane as an elution solvent should be carried out to check the reproducibility of the method. From Table 1, it appears that the recoveries are satisfactory. Most are in excess of 60% and many are in the range of 70 to 100%. These recoveries are much improved over those of 50% for conventional liquid-liquid extractions. The recovery of Picloram is occasionally very low but it has been found that this compound frequently experiences shifts in retention time and is not identified by the instrument.
REFERENCES


<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. SPIKE rises the recoveries expected (in absolute number of

<table>
<thead>
<tr>
<th>ELT1</th>
<th>ELT2</th>
<th>DIAZ2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CAR1</th>
<th>CAR2</th>
<th>DIAZ1</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

| 11.5 | 21.3 | 46.81 | 150 | 250 |

<table>
<thead>
<tr>
<th>Siliex</th>
<th>94.21</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>PCC</th>
<th>Decamela</th>
</tr>
</thead>
<tbody>
<tr>
<td>1234</td>
<td>1235</td>
</tr>
</tbody>
</table>

Reading from both columns, which the readings were obtained. Superscripts "a" denotes an average 0.7% hexane respectively. Superscripts denote column number from ELT1 at solvent blanks of 4% acetone/60% hexane and 30% prop-2-
ELT2 synthesized on Aug, 30th, 1988 and analyzed Aug, 30th, 1989. ELT2 and DIAZ2 synthesized in mid-July of 1988 and analyzed Aug, 30th 1988 and DIAZ2 are blanks prepared using two different lots of diazomethane (Diaz1 and Diaz2 prepared from two different lots of cartridges (see text). Diaz1 and Diaz2 ng/ml when analyzing a spiked water sample. CAR1 and CAR2 are blanks.
Appendix 3. Calibration data for each of the individual pesticides listed in Table 10.

### BASELINE 810 PEAK INTEGRATION REPORT

**Sample:** CAL1

- **Method:** DEMO METHOD 1
- **Acquired:** 24-MAY-1989 13:51
- **Rate:** 1.0 points/sec
- **Duration:** 35.000 minutes
- **Operator:** SAR

**Detector:** detector 1

<table>
<thead>
<tr>
<th>PEK#</th>
<th>ID#</th>
<th>Peak Start (minutes)</th>
<th>Peak End (minutes)</th>
<th>Retention (minutes)</th>
<th>Type</th>
<th>Peak Area (microvolt-sec)</th>
<th>Peak Height (microvolt)</th>
<th>Area Percent</th>
<th>Height Percent</th>
<th>Component Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.000</td>
<td>1.233</td>
<td>1.117</td>
<td>BB</td>
<td>17123.225</td>
<td>3473.7590</td>
<td>5.38</td>
<td>9.78</td>
<td>propoxur</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10.717</td>
<td>11.217</td>
<td>10.967</td>
<td>BB</td>
<td>53770.550</td>
<td>4150.8679</td>
<td>16.88</td>
<td>11.69</td>
<td>carbofuran</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>18.350</td>
<td>18.617</td>
<td>18.500</td>
<td>BB</td>
<td>16624.930</td>
<td>2537.9660</td>
<td>5.22</td>
<td>7.15</td>
<td>cipc</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>20.467</td>
<td>20.750</td>
<td>20.600</td>
<td>BB</td>
<td>25017.265</td>
<td>3943.4437</td>
<td>7.85</td>
<td>11.10</td>
<td>butylate</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>25.550</td>
<td>25.600</td>
<td>25.567</td>
<td>BB</td>
<td>94.771.399</td>
<td>75.101860</td>
<td>0.03</td>
<td>0.21</td>
<td>carbofuran</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>25.983</td>
<td>26.433</td>
<td>26.283</td>
<td>BB</td>
<td>38788.324</td>
<td>2263.7846</td>
<td>12.18</td>
<td>6.37</td>
<td>butylate</td>
</tr>
</tbody>
</table>

**TOTAL**

| Total |                  | 318522.20 | 35517.219 |
### BASELINE 810 PEAK INTEGRATION REPORT

**Printed:** 25-MAY-1989  9:24:34

#### SAMPLE: CAL2

- **Method:** DEMO METHOD 1
- **Acquired:** 24-MAY-1989 14:28
- **Rate:** 1.0 points/sec
- **Duration:** 35.000 minutes
- **Operator:** MAR

#### DETECTOR: detector 1

<table>
<thead>
<tr>
<th>Peak</th>
<th>ID#</th>
<th>Peak Start (minutes)</th>
<th>Peak End (minutes)</th>
<th>Retention (minutes)</th>
<th>Type</th>
<th>Peak Area (microvolt-sec)</th>
<th>Peak Height (microvolt)</th>
<th>Area Percent</th>
<th>Height Percent</th>
<th>Component Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1.083</td>
<td>1.400</td>
<td>1.250</td>
<td>BB</td>
<td>34989.422</td>
<td>5148.6497</td>
<td>5.33</td>
<td>7.36</td>
<td>propoxur</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6.050</td>
<td>11.333</td>
<td>11.050</td>
<td>BB</td>
<td>108946.58</td>
<td>7922.6501</td>
<td>16.59</td>
<td>11.32</td>
<td>carbofuran</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>11.533</td>
<td>12.067</td>
<td>11.783</td>
<td>BB</td>
<td>108336.82</td>
<td>8400.6794</td>
<td>16.50</td>
<td>12.00</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>17.250</td>
<td>18.767</td>
<td>18.600</td>
<td>BB</td>
<td>37662.392</td>
<td>5059.2427</td>
<td>5.73</td>
<td>7.23</td>
<td>captain</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>19.733</td>
<td>20.050</td>
<td>19.883</td>
<td>BB</td>
<td>52769.192</td>
<td>7941.7236</td>
<td>8.03</td>
<td>11.35</td>
<td>cipc</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>20.550</td>
<td>20.867</td>
<td>20.700</td>
<td>BB</td>
<td>51230.498</td>
<td>7706.8813</td>
<td>7.80</td>
<td>11.01</td>
<td>barban</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>26.100</td>
<td>26.600</td>
<td>26.350</td>
<td>BB</td>
<td>79176.438</td>
<td>6242.9911</td>
<td>12.06</td>
<td>8.92</td>
<td>butylate</td>
</tr>
</tbody>
</table>

**TOTAL**

<table>
<thead>
<tr>
<th></th>
<th>656756.86</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>69986.588</td>
</tr>
</tbody>
</table>
# Baseline 810 Peak Integration Report

**Printed:** 25-MAY-1989  9:26:08

**Sample:** CAL3  
**#1 in Method:** DEMO METHOD 1  
**Acquired:** 24-MAY-1989 15:06  
**Rate:** 1.0 points/sec  
**Duration:** 35.000 minutes  
**Operator:** MAR

**Detector:** detector 1

<table>
<thead>
<tr>
<th>PK#</th>
<th>ID</th>
<th>Peak Start (minutes)</th>
<th>Peak End (minutes)</th>
<th>Retention (minutes)</th>
<th>Type</th>
<th>Peak Area (microvolt-sec)</th>
<th>Peak Height (microvolt)</th>
<th>Area Percent</th>
<th>Height Percent</th>
<th>Component Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.100</td>
<td>1.450</td>
<td>1.267</td>
<td>BB</td>
<td>64357.529</td>
<td>9093.2855</td>
<td>6.37</td>
<td>8.56</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10.800</td>
<td>11.433</td>
<td>11.100</td>
<td>BB</td>
<td>171265.63</td>
<td>11932.851</td>
<td>16.95</td>
<td>11.23</td>
<td>propoxur</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>11.583</td>
<td>12.183</td>
<td>11.850</td>
<td>BB</td>
<td>165954.84</td>
<td>12506.246</td>
<td>16.39</td>
<td>11.77</td>
<td>carbofuran</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>13.467</td>
<td>13.933</td>
<td>13.683</td>
<td>BB</td>
<td>113966.48</td>
<td>11905.433</td>
<td>11.28</td>
<td>11.20</td>
<td>carbaryl</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>18.500</td>
<td>18.850</td>
<td>18.650</td>
<td>BB</td>
<td>51895.388</td>
<td>7569.7906</td>
<td>5.14</td>
<td>7.12</td>
<td>captan</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>19.767</td>
<td>20.177</td>
<td>19.933</td>
<td>BB</td>
<td>80907.953</td>
<td>12019.874</td>
<td>8.01</td>
<td>11.31</td>
<td>cipc</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>20.600</td>
<td>20.933</td>
<td>20.750</td>
<td>BB</td>
<td>77265.512</td>
<td>11618.138</td>
<td>7.65</td>
<td>10.93</td>
<td>barban</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>26.117</td>
<td>26.700</td>
<td>26.400</td>
<td>BB</td>
<td>120240.47</td>
<td>9274.4836</td>
<td>11.90</td>
<td>8.73</td>
<td>butylate</td>
</tr>
</tbody>
</table>

**Total:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1010579.0</td>
</tr>
<tr>
<td></td>
<td>106281.05</td>
</tr>
</tbody>
</table>
**BASELINE 810 PEAK INTEGRATION REPORT**

*Printed: 25-MAY-1989 9:27:45*

**SAMPLE: CAL4**

#4 in Method: DEMO METHOD 1  
Acquired: 24-MAY-1989 15:44  
Rate: 1.0 points/sec  
Duration: 35.000 minutes  
Operator: MAR

**DETECTOR: detector 1**

<table>
<thead>
<tr>
<th>PK#</th>
<th>ID#</th>
<th>Peak Start (minutes)</th>
<th>Peak End (minutes)</th>
<th>Retention (minutes)</th>
<th>Type</th>
<th>Peak Area (microvolt-sec)</th>
<th>Peak Height (microvolt)</th>
<th>Area Percent</th>
<th>Height Percent</th>
<th>Component Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.483</td>
<td>1.700</td>
<td>1.283</td>
<td>BB</td>
<td>112821.48</td>
<td>9689.9873</td>
<td>8.12</td>
<td>7.08</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6.067</td>
<td>11.467</td>
<td>11.117</td>
<td>BB</td>
<td>226485.76</td>
<td>15016.796</td>
<td>16.31</td>
<td>10.98</td>
<td>propoxur</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>11.600</td>
<td>12.217</td>
<td>11.883</td>
<td>BB</td>
<td>221103.46</td>
<td>15927.555</td>
<td>15.92</td>
<td>11.65</td>
<td>carbofuran</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>13.517</td>
<td>13.983</td>
<td>13.733</td>
<td>BB</td>
<td>152117.04</td>
<td>15455.486</td>
<td>10.95</td>
<td>11.30</td>
<td>carbaryl</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>16.183</td>
<td>16.833</td>
<td>16.383</td>
<td>BB</td>
<td>222519.67</td>
<td>26994.946</td>
<td>16.02</td>
<td>19.74</td>
<td>ipcl</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>18.517</td>
<td>18.850</td>
<td>18.667</td>
<td>BB</td>
<td>68665.753</td>
<td>9999.2762</td>
<td>4.94</td>
<td>7.31</td>
<td>captan</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>19.783</td>
<td>20.150</td>
<td>19.950</td>
<td>BB</td>
<td>108435.17</td>
<td>16095.640</td>
<td>7.81</td>
<td>11.77</td>
<td>cipc</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>20.617</td>
<td>20.983</td>
<td>20.767</td>
<td>BB</td>
<td>103936.21</td>
<td>15184.481</td>
<td>7.48</td>
<td>11.10</td>
<td>barban</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>22.217</td>
<td>26.733</td>
<td>26.400</td>
<td>BB</td>
<td>172739.05</td>
<td>12389.423</td>
<td>12.44</td>
<td>9.06</td>
<td>butylate</td>
</tr>
</tbody>
</table>

**TOTAL**

| 1388823.6 | 136744.99 |
# BASELINE 810 PEAK INTEGRATION REPORT

Printed: 25-MAY-1989 9:29:02

**SAMPLE:** CAL5

$5$ in Method: DEMO METHOD 1

Acquired: 24-MAY-1989 15:21

Rate: 1.0 points/sec

Duration: 35.000 minutes

Operator: MAR

**DETECTOR:** detector 1

<table>
<thead>
<tr>
<th>PK#</th>
<th>ID#</th>
<th>Peak Start (minutes)</th>
<th>Peak End (minutes)</th>
<th>Retention (minutes)</th>
<th>Type</th>
<th>Peak Area (microvolt-sec)</th>
<th>Peak Height (microvolt)</th>
<th>Area Percent</th>
<th>Height Percent</th>
<th>Component Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.117</td>
<td>1.783</td>
<td>1.333</td>
<td>BB</td>
<td>130282.66</td>
<td>10608.436</td>
<td>7.36</td>
<td>6.30</td>
<td>propoxur</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10.817</td>
<td>11.500</td>
<td>11.133</td>
<td>BB</td>
<td>289499.21</td>
<td>18391.611</td>
<td>16.36</td>
<td>10.93</td>
<td>carbofuran</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>11.617</td>
<td>12.250</td>
<td>11.883</td>
<td>BB</td>
<td>279938.02</td>
<td>19426.348</td>
<td>15.82</td>
<td>11.54</td>
<td>carbaryl</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>13.500</td>
<td>14.000</td>
<td>13.733</td>
<td>BB</td>
<td>193060.67</td>
<td>19088.905</td>
<td>10.91</td>
<td>11.34</td>
<td>ipcl</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>14.733</td>
<td>16.017</td>
<td>16.367</td>
<td>BB</td>
<td>274134.91</td>
<td>33458.474</td>
<td>15.50</td>
<td>19.88</td>
<td>captan</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>17.450</td>
<td>18.867</td>
<td>18.667</td>
<td>BB</td>
<td>84194.553</td>
<td>12580.158</td>
<td>4.76</td>
<td>7.47</td>
<td>cipc</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>19.783</td>
<td>20.133</td>
<td>19.950</td>
<td>BB</td>
<td>135308.52</td>
<td>20153.524</td>
<td>7.65</td>
<td>11.97</td>
<td>barban</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>20.600</td>
<td>20.967</td>
<td>20.767</td>
<td>BB</td>
<td>130276.70</td>
<td>19388.201</td>
<td>7.36</td>
<td>11.52</td>
<td>butylate</td>
</tr>
</tbody>
</table>

**TOTAL**

1769042.3 168312.80
**Propoxur Calibration Report**

Printed: 25-May-1989 10:10:32

**Quant Basis:** Area  
**Curve Type:** Linear  
**Corr. Coef. (r):** 0.99974900

**Rejection Tolerance:** None  
**Weighting:** None  
**Coef. of Determination (r²):** 0.99949807

**Internal Standard:** None  
**Forced Through Origin:** No

Equation: \( \text{Conc} \times (\text{Inj Vol}) = 1.106615E+01 + 1.629073E-03 \times R \)

<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>CAL1</td>
<td>Y</td>
<td>3.8400000E+00</td>
<td>5.3770551E+04</td>
<td>3.946572E+00</td>
<td>-2.70E+00</td>
<td>1.785364E-03</td>
</tr>
<tr>
<td>CAL2</td>
<td>CAL2</td>
<td>Y</td>
<td>3.8400000E+00</td>
<td>1.0894658E+05</td>
<td>3.771001E+00</td>
<td>-1.83E+00</td>
<td>1.762332E-03</td>
</tr>
<tr>
<td>CAL3</td>
<td>CAL3</td>
<td>Y</td>
<td>3.8400000E+00</td>
<td>1.7126562E+05</td>
<td>3.867631E+00</td>
<td>-7.14E-01</td>
<td>1.681598E-03</td>
</tr>
<tr>
<td>CAL4</td>
<td>CAL4</td>
<td>Y</td>
<td>3.8400000E+00</td>
<td>2.2640577E+05</td>
<td>3.800299E+00</td>
<td>1.04E+00</td>
<td>1.695471E-03</td>
</tr>
<tr>
<td>CAL5</td>
<td>CAL5</td>
<td>Y</td>
<td>3.8400000E+00</td>
<td>2.8949922E+05</td>
<td>3.861467E+00</td>
<td>-5.56E-01</td>
<td>1.658036E-03</td>
</tr>
</tbody>
</table>
# Carbofuran Calibration Report


<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>CAL1</td>
<td>Y</td>
<td>4.350000E+00</td>
<td>5.2215465E+04</td>
<td>4.382792E+00</td>
<td>-7.48E-01</td>
<td>2.062716E-03</td>
</tr>
<tr>
<td>CAL2</td>
<td>CAL2</td>
<td>Y</td>
<td>4.350000E+00</td>
<td>1.0833682E+05</td>
<td>4.339452E+00</td>
<td>2.43E-01</td>
<td>2.007628E-03</td>
</tr>
<tr>
<td>CAL3</td>
<td>CAL3</td>
<td>Y</td>
<td>4.350000E+00</td>
<td>1.6559484E+05</td>
<td>4.354009E+00</td>
<td>-9.21E-02</td>
<td>1.970170E-03</td>
</tr>
<tr>
<td>CAL4</td>
<td>CAL4</td>
<td>Y</td>
<td>4.350000E+00</td>
<td>2.2110347E+05</td>
<td>4.327809E+00</td>
<td>5.13E-01</td>
<td>1.967405E-03</td>
</tr>
<tr>
<td>CAL5</td>
<td>CAL5</td>
<td>Y</td>
<td>4.350000E+00</td>
<td>2.7993803E+05</td>
<td>4.363009E+00</td>
<td>-2.98E-01</td>
<td>1.942394E-03</td>
</tr>
</tbody>
</table>

Quant Basis: Area  
Rejection Tolerance: None  
Internal Standard: None  
Curve Type: Linear  
Weighting: None  
Forced Through Origin: No  
Corr. Coef. (r): 0.99996358  
Coeff. of Determination (r²): 0.99992716  

Equation: Conc*(Inj Vol) = 9.641940E+00 + 1.913760E-03 * X
<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>CAL1</td>
<td>Y</td>
<td>4.200000E-01</td>
<td>3.655434E+04</td>
<td>4.313572E-01</td>
<td>-2.63E+00</td>
<td>2.872436E-04</td>
</tr>
<tr>
<td>CAL2</td>
<td>CAL2</td>
<td>Y</td>
<td>4.200000E-01</td>
<td>7.350505E+04</td>
<td>4.137662E-01</td>
<td>1.51E+00</td>
<td>2.856146E-04</td>
</tr>
<tr>
<td>CAL3</td>
<td>CAL3</td>
<td>Y</td>
<td>4.200000E-01</td>
<td>1.139664E+05</td>
<td>4.204495E-01</td>
<td>-1.07E-01</td>
<td>2.763970E-04</td>
</tr>
<tr>
<td>CAL4</td>
<td>CAL4</td>
<td>Y</td>
<td>4.200000E-01</td>
<td>1.521170E+05</td>
<td>4.175969E-01</td>
<td>5.75E-01</td>
<td>2.761032E-04</td>
</tr>
<tr>
<td>CAL5</td>
<td>CAL5</td>
<td>Y</td>
<td>4.200000E-01</td>
<td>1.930606E+05</td>
<td>4.218748E-01</td>
<td>-4.44E-01</td>
<td>2.719352E-04</td>
</tr>
</tbody>
</table>
### ipcl Calibration Report

Printed: 25-MAY-1989 10:41:29

Quant Basis: Area  
Curve Type: Linear  
Corr. Coef. (r): 0.99900766

Rejection Tolerance: None  
Weighting: None  
Coef. of Determination (r²): 0.99961535

Internal Standard: None  
Forced Through Origin: No

Equation: Conc*(Inj Vol) = 3.433245E+00 + 1.422314E-03 * R

<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>CAL1</td>
<td>Y</td>
<td>3.170000E+00</td>
<td>5.1836379E+04</td>
<td>3.086436E+00</td>
<td>2.71E+00</td>
<td>1.528849E-03</td>
</tr>
<tr>
<td>CAL2</td>
<td>CAL2</td>
<td>Y</td>
<td>3.170000E+00</td>
<td>1.1014046E+05</td>
<td>3.201751E+00</td>
<td>-9.92E-01</td>
<td>1.439072E-03</td>
</tr>
<tr>
<td>CAL3</td>
<td>CAL3</td>
<td>Y</td>
<td>3.170000E+00</td>
<td>1.6508522E+05</td>
<td>3.176684E+00</td>
<td>-2.04E-01</td>
<td>1.440165E-03</td>
</tr>
<tr>
<td>CAL4</td>
<td>CAL4</td>
<td>Y</td>
<td>3.170000E+00</td>
<td>2.2251967E+05</td>
<td>3.199261E+00</td>
<td>-9.15E-01</td>
<td>1.424593E-03</td>
</tr>
<tr>
<td>CAL5</td>
<td>CAL5</td>
<td>Y</td>
<td>3.170000E+00</td>
<td>2.7413491E+05</td>
<td>3.146713E+00</td>
<td>7.40E-01</td>
<td>1.445456E-03</td>
</tr>
</tbody>
</table>
# captan Calibration Report

Printed: 25-MAY-1989 10:44:26

**Quant Basis:** Area  
**Curve Type:** Linear  
**Corr. Coef. (r):** 0.99806917

**Rejection Tolerance:** None  
**Weighting:** None  
**Coef. of Determination (r^2):** 0.99614206

**Internal Standard:** None  
**Forced Through Origin:** No

Equation: `Conc*(Inj Vol) = -2.577558E+01 + 1.453958E-02 * R`

<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>CAL1</td>
<td>Y</td>
<td>9.700000E+00</td>
<td>1.6624930E+04</td>
<td>8.637760E+00</td>
<td>1.23E+01</td>
<td>1.458653E-02</td>
</tr>
<tr>
<td>CAL2</td>
<td>CAL2</td>
<td>Y</td>
<td>9.700000E+00</td>
<td>3.7662391E+04</td>
<td>1.043640E+01</td>
<td>-7.06E+00</td>
<td>1.237757E-02</td>
</tr>
<tr>
<td>CAL3</td>
<td>CAL3</td>
<td>Y</td>
<td>9.700000E+00</td>
<td>5.1095387E+04</td>
<td>9.716624E+00</td>
<td>-1.73E-01</td>
<td>1.401859E-02</td>
</tr>
<tr>
<td>CAL4</td>
<td>CAL4</td>
<td>Y</td>
<td>9.700000E+00</td>
<td>6.8665750E+04</td>
<td>9.725959E+00</td>
<td>-2.67E-01</td>
<td>1.412640E-02</td>
</tr>
<tr>
<td>CAL5</td>
<td>CAL5</td>
<td>Y</td>
<td>9.700000E+00</td>
<td>8.4194555E+04</td>
<td>9.587026E+00</td>
<td>1.18E+00</td>
<td>1.440117E-02</td>
</tr>
</tbody>
</table>
### cipc Calibration Report


- **Quant Basis:** Area
- **Curve Type:** Linear
- **Rejection Tolerance:** None
- **Weighting:** None
- **Internal Standard:** None
- **Forced Through Origin:** No

**Equation:** Conc*(Inj Vol) = 1.083643E+00 + 8.945676E-04 * R

<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
</table>
# barban Calibration Report

Printed: 25-MAY-1989 10:58:01

Quant Basis: Area  
Curve Type: Linear  
Corr. Coef. (r): 0.99999249  
Rejection Tolerance: None  
Weighting: None  
Coeff. of Determination (r²): 0.999998498  
Internal Standard: None  
Forced Through Origin: No

Equation: Conc*(Injection/Volume) = 1.499937E+00 + 1.025725E-03 * R

<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>CAL1</td>
<td>Y</td>
<td>1.000000E+00</td>
<td>2.5017266E+04</td>
<td>1.084831E+00</td>
<td>-4.45E-01</td>
<td>1.079235E-03</td>
</tr>
<tr>
<td>CAL2</td>
<td>CAL2</td>
<td>Y</td>
<td>1.000000E+00</td>
<td>5.1230496E+04</td>
<td>1.080166E+00</td>
<td>-1.54E-02</td>
<td>1.054066E-03</td>
</tr>
<tr>
<td>CAL3</td>
<td>CAL3</td>
<td>Y</td>
<td>1.000000E+00</td>
<td>7.7265516E+04</td>
<td>1.076174E+00</td>
<td>3.55E-01</td>
<td>1.048333E-03</td>
</tr>
<tr>
<td>CAL4</td>
<td>CAL4</td>
<td>Y</td>
<td>1.000000E+00</td>
<td>1.0393621E+05</td>
<td>1.080699E+00</td>
<td>-6.47E-02</td>
<td>1.039099E-03</td>
</tr>
<tr>
<td>CAL5</td>
<td>CAL5</td>
<td>Y</td>
<td>1.000000E+00</td>
<td>1.3027670E+05</td>
<td>1.080704E+00</td>
<td>-6.51E-02</td>
<td>1.036256E-03</td>
</tr>
</tbody>
</table>
# butylate Calibration Report

Printed: 25-MAY-1989 11:02:50

**Quant Basis:** Area
**Curve Type:** Linear
**Corr. Coef. \((r)\):** 0.98870927

**Rejection Tolerance:** None
**Weighting:** None
**Coef. of Determination \((r^2)\):** 0.97754602

**Internal Standard:** None
**Forced Through Origin:** No

Equation: \( \text{Conc} \times (\text{Inj Vol}) = 5.315747E+01 + 1.903129E-03 \times R \)

<table>
<thead>
<tr>
<th>Sample</th>
<th>File Name</th>
<th>Valid</th>
<th>Concentration</th>
<th>Response</th>
<th>Calc'd Concentration</th>
<th>% Deviation</th>
<th>Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL1</td>
<td>CAL1</td>
<td>Y</td>
<td>4.070000E+00</td>
<td>3.7807828E+04</td>
<td>5.004425E+00</td>
<td>-1.87E+01</td>
<td>2.691242E-03</td>
</tr>
<tr>
<td>CAL2</td>
<td>CAL2</td>
<td>Y</td>
<td>4.070000E+00</td>
<td>7.9176437E+04</td>
<td>4.076808E+00</td>
<td>-1.67E-01</td>
<td>2.570209E-03</td>
</tr>
<tr>
<td>CAL3</td>
<td>CAL3</td>
<td>Y</td>
<td>4.070000E+00</td>
<td>1.2024047E+05</td>
<td>3.759874E+00</td>
<td>8.25E+00</td>
<td>2.538663E-03</td>
</tr>
<tr>
<td>CAL4</td>
<td>CAL4</td>
<td>Y</td>
<td>4.070000E+00</td>
<td>1.7273905E+05</td>
<td>3.819021E+00</td>
<td>6.57E+00</td>
<td>2.356155E-03</td>
</tr>
<tr>
<td>CAL5</td>
<td>CAL5</td>
<td>Y</td>
<td>4.070000E+00</td>
<td>2.5234703E+05</td>
<td>4.267251E+00</td>
<td>-4.62E+00</td>
<td>2.016073E-03</td>
</tr>
</tbody>
</table>
Conc * Inj Vol

\times 10^2
Appendix 4. The concentrations of the pesticides making up M.O.E. combined standard #1 (MOE 1). The standard was prepared by combining volumes of individual concentrated standards and diluting in acetonitrile.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Propham</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Bux</td>
<td>0.01 ppm</td>
</tr>
<tr>
<td>Cl-Propham</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Barban</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Eptam</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Benomyl</td>
<td>25 ppm</td>
</tr>
<tr>
<td>Butylate</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Diazin</td>
<td>10 ppm</td>
</tr>
</tbody>
</table>
Appendix 5. The concentrations of the pesticides making up M.O.E. combined standard #4 (MOE 4). The standard was prepared by combining volumes of individual concentrated pesticide standards in acetonitrile and diluting in water.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran</td>
<td>0.96</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>1.09</td>
</tr>
<tr>
<td>Propham</td>
<td>1.06</td>
</tr>
<tr>
<td>Bux</td>
<td>0.02</td>
</tr>
<tr>
<td>Cl-Propham</td>
<td>0.98</td>
</tr>
<tr>
<td>Barban</td>
<td>1.08</td>
</tr>
<tr>
<td>EPTC</td>
<td>1.00</td>
</tr>
<tr>
<td>Butylate</td>
<td>1.02</td>
</tr>
<tr>
<td>Diallate</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Appendix 6. The concentrations of the pesticides making up M.O.E. combined standard #7 (MOE 7). The standard was prepared by combining volumes of individual concentrated standards in acetonitrile and diluting in water.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminocarb</td>
<td>1.04</td>
</tr>
<tr>
<td>Propoxur</td>
<td>0.96</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>1.09</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>1.06</td>
</tr>
<tr>
<td>Propham</td>
<td>1.06</td>
</tr>
<tr>
<td>Captan</td>
<td>4.85</td>
</tr>
<tr>
<td>CI-Propham</td>
<td>0.98</td>
</tr>
<tr>
<td>Barban</td>
<td>1.08</td>
</tr>
<tr>
<td>EPTC</td>
<td>1.00</td>
</tr>
<tr>
<td>Butylate</td>
<td>1.02</td>
</tr>
<tr>
<td>Diallate</td>
<td>1.00</td>
</tr>
</tbody>
</table>
REFERENCES


73. Yago, L., Am. Lab., October, 1985, 118.


