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DICHLOROBENZYL ALKYL ETHERS AS STANDARDS IN ENVIRONMENTAL ANALYSIS USING GAS CHROMATOGRAPHY

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DEDICATION

For my parents, with love.

ABSTRACT

The ethyl-hexadecyl members of a homologous series of 2,4-dichlorobenzyl alkyl ethers were prepared using the Williamson Ether Synthesis and purified by chromatographic methods. Temperature programmed gas chromatographic retention indices were calculated for a range of chromatographic conditions of column stationary phases, temperature programme rates, and starting temperatures for wall-coated open tubular columns. The n-alkanes were used as retention index standards. The DCBE retention indices were used to calibrate a gas chromatographic identification system for organochlorine pesticides and polychlorinated biphenyls and their utility was demonstrated in the identification and measurement of organochlorine residues in a series of fish oils from North Atlantic catches.

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INTRODUCTION

1.1 HISTORY OF GAS CHROMATOGRAPHY AND DEVELOPMENT OF OPEN TUBULAR COLUMNS

1.1.1 Historical Background to Gas Chromatography

As a result of its resolving power, speed and sample size requirement, gas liquid chromatography (GLC) is one of the most widely used techniques in organic analytical chemistry.

The concept of gas chromatography was first suggested by Martin and Synge in 1951[1] but the theory was not demonstrated in practice until 1952 when James and Martin subjected a fatty acid mixture to a relatively crude separation and monitored the results by titration.[2]

The technique gained acceptance during the 1950's and 1960's following this pioneering work, and the development of reliable equipment for reproducible quantitative analysis favoured its use for the analysis of a wide variety of organic compounds.

1.1.2 Theoretical Consideration of Gas Chromatography

The resolution of a mixture in gas chromatography is dependent on the differential partition of the components between a stationary phase, usually a non-volatile liquid, and a mobile phase, which in this case is a gas.

Consider a mixture of two compounds, one of which is insoluble in the liquid phase and one of which is equally soluble in the liquid and the gas phase. Injection of the mixture results in the molecules of the first material passing through the column at the same rate as the carrier gas, whereas the molecules of the second component spend an equivalent amount of time in the liquid and gas phases and require twice the time to pass through the column. The two compounds emerge from the column at different times and hence will be separated.

In an actual separation the components spend an amount of time in the column proportional to the solubility of each component in the liquid phase. A sample passing through a column will distribute itself between the vapour and condensed (stationary) phase in a ratio described by the partition coefficient (K) which is shown in equation 1 below.

$$K = \frac{C_s}{C_M} \qquad (1)$$

where C_s is the concentration of the sample in the stationary phase, C_M is the concentration of the sample in the mobile phase, and K, the partition coefficient, is a true equilibrium constant. The higher the value of K, the longer the retention time of the sample on the column. Separation occurs when the partition coefficients of the samples differ and when the peak overlap is minimized. This latter factor is dependent on the control of zone spreading, or the minimization of the band width, i.e. peak width which is described using the concept of the height equivalent to a theoretical place (HETP) or plate height (H).

The simplest and most widely used equation describing this concept is the Van Deemter plot[3] which provides an approximate relationship between the flow rate and plate height for a packed column and is shown below in equation 2.

$$H = A + \frac{B}{V} + CV \qquad (2)$$

where A, B, C are constants derived from a consideration of eddy differences, longitudinal diffusion and mass transfer in the column and V is the carrier gas velocity.

The A term is independent of the gas flow velocity but is related to the particle size, geometry and packing in the column. B is related to the diffusion coefficient of molecules in the mobile phase. The C term describes the kinetics of mass transfer between the mobile and stationary phases. Analysis of the equation showed that the most efficient columns would be packed with small spherical particles and a thin, evenly applied stationary phase.

Since this work the use of gas chromatographic techniques expanded rapidly and the apparatus became cheaper and more readily available. Until the 1970s packed columns of 1-2m in length and 1-4mm diameter were the most popular columns used but it was observed that they had a number of limitations in terms of efficiency, longevity and reproducibility in the packing. These limitations have been reviewed by Jennings,[4] and the major points are summarised below:

1. The conditions in a packed column may only be regarded on the macro scale and the micro-environments which each molecule encounters as it passes through the column differ; i.e. it is only possible to consider the column on a large scale and it is impossible to examine the individual molecular pathways and environments throughout the columns.
2. Under the chromatographic conditions employed (usually elevated temperatures) the stationary phase is liquid and after temperature cycling it may flow. Points of contact between particles and/or the column wall may become centres of thicker stationary phase which may retard the solute molecules causing band broadening with a subsequent decline in separation efficiency.
3. The multitude of flow paths through the column packing allows a range of possible retention times for the solute again resulting in peak broadening.

4. Most column packings have poor heat transfer properties and it is possible to have a range of temperatures across the column.

It is these factors which give packed columns their inherent low efficiencies but the problems have largely been overcome by the development of the open tubular column.

1.1.3 Development of Open Tubular Columns for Gas Chromatography

The possibility of using empty (open tubular) columns with liquid coated walls was raised by Martin as early as 1956 but their practical application followed the theoretical and practical work of Golay[5] in 1959. He arrived at the application of a liquid coating to tubes of capillary dimensions on purely theoretical grounds through an investigation of zone spreading as a result of packing particles and pressure drops across the column. Examination of the diffusion of a gas in an empty tube led to the theory which predicted a chromatographic performance significantly better than those available using packed columns. The theory is stated in a simplified form in equation 3.

$$H = \frac{B}{V} + C_s V + C_M V \qquad (3)$$

where C_s corresponds to C in equation 2, C_M represents diffusion resistance in the gas phase, V is the flow rate, and the other terms are as previously defined in equation 2.

Comparison with equation 2 shows there is no term related to the packing (the A term in equation 2) and a gas diffusion coefficient is present. Golay also showed the differences between the mechanisms of diffusion processes in both columns and proved that mass transfer played

a greater role in packed columns rather than open tubular columns.[6]

The advantages of wall coated open tubular columns (WCOT) have been reviewed by Jennings,[4] and are summarised below:

1. The lower pressure drop across the open tubular column permits the use of longer columns.
2. The stationary phase may be applied as a thinner, more uniform film on the column walls.
3. All gas flow paths are essentially the same length.
4. There is a more uniform temperature distribution across the column.

As a result of the above factors, in WCOTs solute molecules show a much narrower range of retention times, improving the peak shape, separation efficiency and sensitivity. The major disadvantages with WCOT columns are a reduced sample capacity and they are less robust in use compared to packed columns.

1.1.4 Development of Materials for Column Construction

Early workers with WCOT columns used a wide variety of materials for column construction including plastic (polyamides), stainless steel, copper and other materials. However, all these materials possessed drawbacks such as thermoplasticity, catalytic activity and oxidation at high temperatures in the metal columns. The early developments in WCOT columns have been reviewed by Ettre.[7]

In 1960 Desty developed a machine for drawing glass capillaries[8] and since then glass has become the material most commonly used for column construction. Although glass was superior to the materials already mentioned it still had a number of drawbacks, in particular its fragility and the difficulty in completely deactivating the glass surface. This activity is primarily due to metal ions and metal ion

impurities present in the glass acting as Lewis acid sites and free surface hydroxyl groups hydrogen bonding with suitable solutes.[9] Boron impurities in the glass can also act as Lewis acid sites.[10]

Several approaches have been used to overcome this problem including leaching the metal ions from the glass with HCl[11] and silylating the surface silanol groups to deactivate them.[12] An alternative was to use a very high purity glass such as quartz or fused silica materials, commonly used in the fibre optics industry. The use of fused silica was first reported by Dandeneau and co-workers,[9,13] who compared several different types of glass for column construction. They found fused silica was intrinsically more inert than the other glasses, it was more flexible because of the thinner walls and it had a higher tensile strength than the other materials. Being inherently straight it did not require flame straightening which may regenerate silanol groups and cause metal ion migration. The method of production of the fused silica by hydrogen flame decomposition of silicon tetrachloride also meant there was less than one part per million of metallic impurities present.

However, the columns needed to be protected from abrasions by means of a polyimide sheath and the surface of the column modified before coating with a stationary phase using high temperature silylation.[14] Recent developments in the technology include the ability of manufacturers to provide columns of consistent quality both for polar and non-polar separations. The columns which are commonly in use today have dimensions of between 10-50 metres in length and internal diameters of 250-540 m.

1.1.5 Stationary Phases for Open Tubular Columns

A great variety of stationary phases were developed for use with packed columns and this range was largely because they were being used to

compensate for the lack of efficiency of the packed columns, with selectivity for solute pairs being achieved by means of the stationary phase rather than the column. Very few of these phases have been successfully applied in open tubular gas chromatography since the performance requirements for high efficiency and sensitivity were so demanding that most of the packed column phases commercially available were not suitable.

Blomberg[15] characterized the performance of an open tubular column using four factors: efficiency, deactivation, stability and precision and reviewed the influence of the stationary phase on these factors. He suggested that the column should be deactivated, the stationary phase should not decompose thermally or otherwise and it should not be displaced from the surface or form droplets.

Silicone based polymers are the most widely used stationary phases for WCOT columns today. They are thermally stable, show low temperature dependence of physical properties such as viscosity and they have a good film forming ability. The polymers also demonstrate a high permeability to solute vapours due to the high chain mobility, which allows openings which in turn permit easier vapour diffusion. The silicones most commonly used are the methyl silicone gums with a 6-8% incorporation of phenyl groups to improve the low temperature performance.

A further improvement of silicone stationary phases was the process of cross-linking which effectively immobilises the stationary phase and greatly decreases the tendency to lose viscosity with increasing temperature during temperature programming. Cross-linking the stationary phase also makes the film relatively resistant to 'wash out' by organic solvents.

The choice of stationary phase ultimately depends on the sample to

be analysed and the desired resolution of the components. Initially it was assumed that only non-polar stationary phases should be used for non-polar compounds and polar stationary phases for polar compounds. However, with new surface deactivation techniques, polar solutes can be adequately analysed on non-polar stationary phases but in most cases the resolution of complex mixtures of polar compounds is better on polar phase columns. The non-polar methyl siloxane gum phases (OV-1, SE-30, SE-52, SE-54) provide the most uniform and stable stationary phase films available. The characterization of stationary phases will be discussed in section 1.4.8. A review of stationary phases is also given by Ettre.[16]

1.1.6 Alternatives to Wall Coated Open Tubular Columns

Variants of conventional wall-coated open tubular columns include porous layer open tubular columns (PLOT columns) and support-coated open tubular columns (SCOT columns). In these the stationary phase is coated on a porous, generally particulate layer on the column inner wall, thus increasing the column surface area.

In general, SCOT columns have properties intermediate between those of packed columns and wall coated open tubular columns such as an increased sample capacity compared to open tubular columns. The principle disadvantages of PLOT and SCOT columns are practical restrictions on length and lower efficiencies per unit length compared to the open tubular columns.

1.2 THE GAS CHROMATOGRAPH

As the efficiency and reproducibility of GC columns improved so has the performance of the gas chromatograph itself. Manufacturers have developed GCs dedicated to open tubular columns rather than modify packed column GCs. The requirements for these dedicated GCs include improved thermal stability, reproducible linear temperature programming and low thermal gradients in the oven. As a result, however, of the lower sample capacities of WCOT columns, attention must be focussed on considerations of injection and detection techniques, the most important of which are discussed below. A review of the requirements and developments of GCs for WCOT columns is given by Lee et al.[17]

1.2.1 Injection Systems for WCOT Gas Chromatography

The design and performance of the injection system in a gas chromatograph is vital to the overall chromatographic performance in terms of peak shape, resolution and reproducibility. The utility and, hence, selection of an injector depends on the sample concentration, volatility, boiling point, thermal and chemical stabilities. The three most commonly used injection systems are the splitter injector, the splitless injector and the cold on-column injector.

(a) Splitter Injector

The splitter injector is a flash vaporisation injector that allows a small fraction of the volatilized sample to enter the column while venting the rest to waste. The basic design was introduced by Desty et al[18] in 1959 and its use has been reviewed by Schomburg.[19] The design requires minimum band spreading, maximum chemical inertness, minimum contamination, linear and reproducible sample splitting and a high temperature operating range. An assessment of the technique has

been reported by Grob.[20]

The advantages of split sampling are:

i) Negligible initial solute molecular zone spreading.

ii) Split ratios can be easily adjusted for sample volume concentration.

iii) Excellent retention time reproducibility.

iv) Compatible with both isothermal and temperature programmed operation.

v) Sample dilution is not required.

The disadvantages of the technique include:

i) It is discriminatory, i.e. poor recovery for low volatility samples.

ii) Trace analysis is difficult.

iii) Large consumption of carrier gas.

iv) The quantitation, precision and accuracy are heavily dependent on injection repeatability, injection technique and split reproducibility.

The splitter injector may be best described as a vaporising injector in which the evaporated sample is mixed with the carrier gas and divided into two streams, one exiting to the column and the other vented to the atmosphere. The appropriate column flow may be achieved by injecting 0.1 to 2.0 µl with a 'split' ratio between 1:20 and 1:200.

(b) Splitless Injector

The splitless injector is very similar to the splitter injector and differs only in the geometry of the sample vaporisation chamber. The technique was developed by Grob and Grob[21,22,23] and involves evaporation of the sample using a high injector temperature and a low column temperature to utilize the 'solvent effect'[21,23]. This 'refocuses' the sample at the top of the column which is considerably cooler than the vaporisation chamber.

In this system the sample is injected into a vaporisation chamber

through which carrier gas is flowing and after a preset time (30-90 sec) a purge function is activated by redirecting the gas flows such that one portion continues to flow through the column to serve as carrier gas while the other sweeps residual volatiles from the chamber to atmosphere. The advantages of splitless injectors are:

i) Analysis of dilute samples without preconcentration is possible.

ii) Sample components eluted near the solvent tail have narrow peak widths due to the 'solvent effect'.

iii) Large samples can be injected using a slow injection, since the initial band width is minimized by the solvent effect.

iv) Thermally labile compounds can be analysed because rapid vaporisation of the sample is not required and consequently, relatively low injection front temperatures can be used to minimize sample degradation.

v) The technique is readily automated.

The disadvantages of splitless injection are:

i) The quantitative results depend on the injection techniques, for example temperature and speed.

ii) Solvent selection is important as the column temperature must be below the solvent boiling point.

iii) A retention time 'shift'[24] may be observed for compounds eluting near the solvent tail.

iv) Solutes eluting before the solvent will not be separated efficiently.

v) The technique is limited to a restricted boiling range.

vi) A calibration run is generally required for accurate quantitation.

(c) Cold On-column Injector[25,26,27]

Cold on-column injection allows the injection of a sample directly into the inlet of the column, eliminating the possibility of loss during

vaporisation and transfer.

The advantages of the cold on-column injection technique include:

i) The avoidance of thermal degradation.

ii) No discrimination against high boiling samples.

iii) There is no carrier gas waste.

iv) Quantitative analysis of relatively involatile samples is possible.

The disadvantages of this technique are:

i) Sample clean up procedures are vital, particulate and non-volatile contaminants must be removed to prevent column damage.

ii) A column diameter of less than 0.2mm is difficult to use because of constraints of syringe and injector construction.

iii) The condensed solvent may strip the stationary phase from the front of the column causing a rapid loss of efficiency[23] although this problem has largely been overcome by the use of chemically bonded stationary phases.

A review of the injection systems has been given by Schomberg[29] and in reference 17. In general terms, cold on-column injection allows the possibility of excellent precision and accuracy for quantitation of wide boiling range samples whereas splitless injection gives less precise results[30] but allows easier automation.

1.2.2 Detection Systems for WCOT Gas Chromatography

The use of open tubular columns in gas chromatography also demands rigorous detector design, including low detector dead volume, sensitivity, baseline stability and linear dynamic range. WCOT columns, in general, operate in the low mass flow rate or concentration range of the detector and in some cases outside the linear dynamic range of detectors causing peak distortion and quantitation errors. Some of

the more commonly used detectors are discussed below.

(a) Flame Ionization Detector (FID)

This is the most commonly used detector in gas chromatography. The basis of operation is that gases burning in a hydrogen flame contain an appreciable concentration of free electrons. Burning of organic compounds increases the conductivity and this increase in conductivity is proportional to the sample concentration. The development and use of FIDs has been discussed by McWilliam[31] and Harley et al.[32]

The advantages of FIDs are:

i) Good sensitivity to organic carbon containing compounds.

ii) Good linear range (concentration range over which detector response constant).

iii) Ease of use.

iv) Relatively insensitive to small flow rate changes in temperature programmed operation.

v) Low dead volume effects.

vi) Fast response time constant.

The major disadvantages of FIDs include:

i) Little or no response to compounds such as the noble gases and CO_2, H_2O, H_2S.

ii) Relatively insensitive.

iii) It is a destructive detector.

iv) Detector response is very dependent on sample structure and the presence of heteroatoms (oxygen, sulphur and halogens decrease the response).

v) Excessive amounts of halogenated solvents (1 µl) produce flame instability.

(b) Electron Capture Detector (ECD)

The electron capture detector is used to selectively determine compounds with a high electron affinity such as organochlorine pesticides, polychlorinated biphenyls, drugs and their metabolites. This detector was first developed by Lovelock and Lipsky[33] and operates by the generation of a standing current between two electrodes, one of which is a β source (usually Nickel-63). When a compound with a high electron affinity enters the detector a reduction occurs in the standing current.

The advantages of the electron capture detector are:[34]

i) It is non-destructive.

ii) It is highly sensitive.

The disadvantages of the detector are:

i) The detector response is strongly dependent on the sample concentration and its electron affinity.

ii) It has a lower linear range than the FID.

iii) A small detector is required to maximise sensitivity and minimize dead volume.

(c) Mass Spectrometer

The combination of WCOT column gas chromatography with a mass spectrometer is regarded as one of the most powerful tools for the analysis of volatile samples. The mass spectrometer has both high selectivity and sensitivity and it can be used as a specific detector by means of a selected monitoring mode. Using the scanning mode for qualitative analysis means it has the highest information content of all identification and structure elucidation methods for organic compounds. The major disadvantages of the mass spectrometer are its low linear dynamic range which is limited by the signal to noise ratio and the high

cost. The practical aspects of the technique have been reviewed by McFadden.[35]

(d) Other Detectors

There is a wide range of alternative detectors including the flame photometric detector, the nitrogen phosphorus detector and the photo-ionization detector. Details of these detectors are given by Lee et al.[17]

1.2.3 Temperature Programmed vs Isothermal Operation in WCOT Gas Chromatography

The use of open tubular columns in gas chromatography has meant that more complex samples may be analysed than was previously possible with packed columns. However, this has resulted in samples containing compounds with a wide boiling range and chromatographing such a mixture at a constant temperature gives narrow initial peaks but the later eluting peaks are very wide and shallow giving problems with quantitation and long analysis times. Some form of temperature programming is thus required if the full power of WCOT is to be realised.

The theoretical aspects of the linear temperature programming have been discussed by Habgood and Harris,[36] Giddings[37] and Grant and Hollis.[38] The technique has a wide application in gas chromatography particularly environmental analysis. The technique allows faster analysis times, improved peak shape and quantitation for high boiling samples.

1.2.4 Quantitative Analysis in WCOT Column Gas Chromatography

Open tubular column gas chromatography overcomes several major problems associated with packed columns such as surface adsorption,

catalytic decomposition and limited resolving power. However, the lower sample capacities and the complex injection and detection techniques mean that quantitation requires a more rigorous approach.

For accurate quantitation the peak size must be measured using either peak height or peak area and then standardised using some known sample. This is generally performed using either area normalisation, internal standard, external standards or standard addition.[17,76]

The simplest technique is area normalisation in which the percentage of each component in the chromatogram is calculated. However, if detector responses are not equal a response factor is required for normalisation. This method is successful for relatively simple mixtures but it is not suitable for mixtures where there are unresolved compounds. It is also unsuitable for use with selective detectors.

The internal standard method has the advantage that it does not require complete resolution, elution and detection of all sample components, only that there is complete resolution of the peak(s) of interest and the internal standard. The internal standard must fulfil a number of important criteria which are discussed in Section 1.6. A calibration curve is usually calculated using standard solutions containing the component(s) of interest at several concentration levels but all containing the same concentration of internal standard. This method minimises quantitative errors due to sample preparation and injection, allows the quantitation of more than one component in the sample matrix and only requires resolution of the sample component and the internal standard.

In external standardisation, peak areas or peak heights of unknowns are compared with those of a series of unknown (or external) standards containing the components of interest. The method requires that the

external standards have the same concentration levels as the components of interest, that chromatographic conditions are the same, that the concentration range covers the entire range expected in the unknown, and that calibration curves are obtained using the same volume of standards. This technique is usually applied when reproducible sample sizes can be injected onto the column. In open tubular column GC it is difficult to obtain a high degree of precision because of the small sample size and injector discrimination, a difficulty which may be overcome by on-column injection.

The standard addition method is both external and internal standardisation. The unknown sample is first chromatographed. Known amounts of the pure component are then added to a known volume or quantity of the unknown sample and the solution chromatographed. One of the original sample components may act as a reference or a pure component may be added as an internal standard. This method has the advantage that matrix effects are cancelled.

1.3. QUALITATIVE ANALYSIS IN GAS CHROMATOGRAPHY

1.3.1 General Methods of Qualitative Analysis

The retention of a solute by the liquid phase in gas chromatography is a physical characteristic of its chemical nature and can be used to aid compound identification. The fundamental prerequisite for successful identification is the adequate separation of each of the components into discrete peaks.

The most commonly used identifier is the retention of each component, either the retention time or retention volume. The retention time is the time taken for a peak to pass through the column from injector to detector under defined instrument conditions. The retention volume is a product of the carrier gas flow rate and the retention time, which equates the volume of gas required to elute a component from the column.

The advantage of the retention volume is that it is independent of a potentially fluctuating column variable, the flow rate. The concept was introduced by Littlewood[39] to eliminate the influence of temperature, pressure changes, flow rate and the quantity of stationary phase. However, although it is a theoretically exact value, it is not used for practical identification, partly because its determination is difficult and partly because it produces reliable values only under rigorously controlled conditions which are not always achievable on commercially available instruments. Retention times are easier to measure and are thus the most commonly used retention parameter.

In general, absolute retention values (either times or, specific volumes) are rarely used as these are dependent on reproducible column parameters and operating conditions. Retention is more commonly measured relative to a standard compound or series of compounds since

this is independent of flow rate, pressure changes and, where similar compounds are involved, will only have a small temperature dependence.

The use of relative retention was first described by James and Martin[2] but the initial drawback in using the system was the lack of a universal reference system for all compounds. Attempts have been made to standardise the systems used and these are described below.

1.3.2 Theoretical Nonane Method (Rx9)

Smith[40] introduced the theoretical nonane value for relative retention with reference to a single standard substance. This value is determined by plotting log t versus the number of carbon atoms for a series of n-alkanes and marking the point where the best fitted line calculated from experimentally determined points, intersects the vertical line corresponding to nonane. The experimental data are referred to the standard retention time represented by this 'theoretical' nonane value. The sample is analysed with an n-alkane and the relative retention with reference to theoretical nonane is given by:

$$R_{x9} = R_{xa} \cdot R_{N9} \qquad (4)$$

where R_{xa} is the retention time of the sample and R_{N9} is the retention time of nonane.

This approach has been reviewed by Evans and Smith.[41,42,43] The accuracy of the determination is dependent on the distance of the primary standard from n-nonane and the difficulty in describing the temperature dependence of the results.

An alternative to this approach is the retention index system, described in the following section.

1.4. RETENTION INDEX SYSTEMS

1.4.1 Kovats' Retention Index System

The retention index (RI) system described by Kovats[44-47] refers the retention to a series of standards rather than a fixed standard. The approach is based on the relationship between the carbon number of a homologous series and the logarithm of the retention time or retention volume demonstrated by James and Martin.[2]

In relative retention schemes the retention of a substance is usually related to one standard. However, in the retention index scheme the retention behaviour of the sample is based on a uniform scale determined by a series of closely related standard substances. This has the advantage that the retention of any eluate is measured on a scale provided by the chromatogram itself; the fixed points are the elution maxima of the individual members of the homologous series of n-alkanes.

At any column temperature or for any given stationary phase, the points corresponding to the elution maxima of the general formula C_ZH_{2Z+2} are always defined as 100Z

$$I_T^{st.ph}\ (n\text{-}C_ZH_{2Z+2}) = 100Z \qquad (5)$$

where $I_T^{st.ph}$ is the retention index on a stationary phase at a defined column temperature (T) and $n\text{-}C_ZH_{2Z+2}$ is an n-alkane.

In isothermal gas chromatography the retention index of any substance X is found by the logarithmic interpolation between two bracketing alkanes using equation 6:

$$I_T^{st.ph}\ (X) = 100\left[\frac{\log V_R(X) - \log V_g(A_Z)}{\log V_g(A_{Z+1}) - \log V_g(A_Z)} + Z\right] \qquad (6)$$

where $Vg(A_Z)$, $Vg(A_{Z+1})$, and $Vg(X)$ are the retention volumes of an alkane with Z carbon atoms, Z+1 carbon atoms and the unknown, respectively. Equation (6) is dependent on the condition $Vg(A_Z) < Vg(X) < Vg(A_{Z+1})$. In equation 6 the specific retention volume may be replaced by the retention time (T) if the measurements are obtained under identical conditions or if the standards are mixed with the unknowns prior to analysis. Equation 6 then becomes:

$$I^{st.ph}_{temp}(X) = 100\left[\frac{\log T(X) - \log T(A_Z)}{\log T(A_{Z+1}) - \log T(A_Z)} + Z\right] \qquad (7)$$

The retention index expresses the retention of a compound relative to the n-alkanes analysed under identical isothermal conditions and may be defined as the carbon number of a hypothetical n-alkane multiplied by 100 which would have exactly the same retention characteristics as the compound of interest. The conversion to logarithms makes the arbitrarily chosen scale linear as the logarithms of the retention times of the n-alkanes increase linearly with increasing chain length.[48] The history and development of the retention index scheme is described in references 48-51 and has been extensively reviewed by Budahegyi et al.[52]

Retention indices have been used for peak identification, stationary phase classification, physico-chemical characterization and structure-retention studies. Some of these applications will be discussed in later sections.

1.4.2 Retention Indices in Temperature Programmed Gas Chromatography

The analysis of wide boiling range mixtures by GC resulted in the introduction of linear temperature programming c.f. section 1.2.3, which caused numerous theoretical and practical problems for peak identification.

Van den Dool and Kratz[53,54] reported an approximate linear relationship between the elution temperature T_R of an n-alkane and the chain length:

$$I^{st.ph}_{TPGC} = 100 \frac{T_R(X) - T_R(A_Z)}{T_R(A_{Z+1}) - T_R(A_Z)} + Z \qquad (8)$$

where $T_R(A_{Z+1})$, $T_R(A_Z)$, $T_R(X)$ are the elution temperatures of the n-alkanes and the unknown sample X. Equation 8 must fulfil the condition $T_R(A_Z) \leq T_R(X) \leq T_R(A_{Z+1})$.

The values of the retention indices in equation 8 are equivalent to the isothermal RI provided the indices measured are independent of temperature.

The inter-conversion of isothermal and temperature programmed indices has been considered by many workers. For example, Giddings[55] established that relative retention times for a temperature programmed chromatogram are approximately the same as those for isothermal conditions provided the condition shown in equation 9 is met:

$$T = 0.92\ T_R \qquad (9)$$

where T is the isothermal column temperature.

A simpler equation was derived by Guiochon[56] shown below in equation 10:

$$T = T_R - 20 \qquad (10)$$

However, these methods do not account for the influence of initial column temperature, temperature programme rate and column dimensions on

retention indices. Curvers[57,58] found that direct conversion of programmed indices into isothermal indices was not possible but could be achieved by incorporating the column dead time and distribution coefficients into the calculation.

Golovnya and Uraletz[59] calculated the temperature programmed retention indices taking into account the isothermal index temperature dependence, and they stated that the temperature programmed retention index was a complex function of experimental conditions, but did not account for this in their equation. Grant and Hollis[38] reported a method for temperature programmed retention indices for aromatic hydrocarbons and found a linear relationship between the boiling point and elution time provided the initial column temperature was low.

Erdey et al[60] showed that retention indices could be calculated using equation 11:

$$I_{TPGC}^{st.ph} = A + \frac{B\ln(\frac{T_R+C}{T_0+C})}{T_R - T_0} \qquad (11)$$

where A, B and C are constants, T_0 is the initial temperature and T_R is the elution temperature.

Majlat et al[61] discussed a method of calculation for temperature programming where there are separate periods of isothermal and programmed conditions. This method was further developed for more complex programming by An Zhu.[62]

1.4.3 Effect of Chromatographic Parameters on Retention Indices

The magnitude of the retention index is independent of the following parameters reviewed in reference 52:

(a) The quality and chemical nature of the solid support and/or the quality of the open tubular column, assuming the column does not show any wall effects. If the solid support or column wall is not inert, solute adsorption processes can occur, so increasing the retention of the sample and hence the retention index. These retention index values are only reproducible if the support is inert or the column properly deactivated.

(b) The chemical nature and physical parameters of the carrier gas provided they remain constant.

However, the magnitude of the retention index is dependent on:

(a) The chemical nature of the solute. Only one retention index can correspond to a given compound but a number of compounds may have the same retention index. Thus unambiguous peak identification can only be achieved by comparing data from more than one stationary phase with widely different polarities so that the retention characteristics are widely different.

(b) The chemical nature of the stationary phase. In open tubular columns the uniformity and film thickness of the stationary phases is much more consistent than with packed columns.[63] The polarity of the stationary phase has the greatest effect on retention indices (see also section 1.4.8).

(c) Column temperature. It was originally assumed that there was a linear relationship between retention index and column temperature. However, the relationship is now more accurately described by an Antoine type hyperbolic approximation shown in equation 12:[64]

$$I^{st.ph}(T) = A + \frac{B}{T+C} \qquad (12)$$

where A, B and C are constants and T is the column temperature for isothermal operations. For non-polar compounds the relationship is almost completely linear and can have significant linear portions, the length being dependent on the polarity of the compounds, the polarity of the stationary phase and the column/compound interactions.

1.4.4 Sources of Error in Retention Index Measurement

The uses of retention indices are limited by the accuracy and precision with which they can be calculated and in their early use this accuracy was dependent on the gas hold up time or dead time. The effect of this value on the accuracy of retention index determination was reviewed by Kaiser.[65] Methods of dead-time calculation were reviewed by Wainwright and Haken[66] and Smith et al.[67]

The measurement of retention indices in WCOT columns, however, is less dependent on the gas hold-up time, as this is small compared with the retention times of the samples, and constant, thus cancelling in most calculations. Ballschmiter[68] states that the dead time is only dependent on the flow rate of the mobile phase and therefore adds as a constant factor, allowing the use of absolute rather than adjusted times. Chien et al[63] showed it was possible to use such absolute data over a prolonged period of time without affecting accuracy or precision. Rijks[69] studied the factors important in random error and reported that column temperature, carrier gas velocity, and time measurement were the most important factors. Vernon and Suratman[70] found that variations in retention indices increased with stationary phase polarity and changing alkane concentration. The effect was more pronounced in polar phases than non-polar phases.

1.4.5 Linearity of Retention Time versus Carbon Number Plot

Almost all methods for the calculation of retention indices are based on the use of equation 13:

$$\log t_R' = aI+b \qquad I = 100Z \tag{13}$$

where t_R' is the adjusted retention time and a and b are constants. This assumes a linear relationship between the logarithm of the adjusted retention time and carbon number. There is evidence, however, both experimental and theoretical that this is not the case.

Extensive evidence has been provided by Haken and co-workers[71] showing non-linearity of the plot for n-alkanes, n-alcohols, n-aldehydes and acetates and methyl ketones. The evidence has also been reviewed by Smith, Haken and Wainwright.[72]

Golovnya and Misharina[73] proposed an equation for calculating the partial molar free energy of sorption of a compound or its structural fragments from the retention index value. It was found to depend on the molecular structure, the stationary phase polarity and the nature of intermolecular interaction. The free energy equivalent of one index unit (ΔGi.u.) was shown to depend on the stationary phase polarity and column temperature and could be calculated using an equation developed by Golovnya[74] for n-alkanes:

$$\Delta Gi.u. = -0.023RT\ (\log Vg_Z - \log Vg_{Z-1}) \tag{14}$$

where Vg_Z and Vg_{Z-1} are the retention volumes of n-alkanes containing Z and Z-1 carbon atoms respectively; T is the column temperature in degrees K and R is the universal gas constant.

Golovnya and Grigoreva[75] showed that the values of free energy contributions per methylene unit in n-alkanes is not constant for both polar and non-polar stationary phases in virtually all the series members and that the largest change was observed for the first methylene group. A more complex dependence was observed in homologous series where a functional group or functional groups were present. The free energy contribution for the first methylene group was less than that for the second. The further removed the methylene group was from the functional group the less the effect of the functional groups and the closer the values of ΔG for the series.

The authors postulated the deviation from linearity was due to van der Waals interactions differing for the functional groups bound directly to a methylene radical compared with those having 3-4 methylene groups. The compounds containing two adjacent methyl groups as substituents showed deviations due to the increasing energy of the van der Waals interactions.

1.4.6 Correlation of Physico-Chemical Properties and Retention Index

The relationship existing between the specific retention volume and retention index is indicative of the relationship between retention index and other thermodynamic properties of a compound. A knowledge of such relationships is valuable in the examination of other solute properties. A detailed discussion of the thermodynamic basis of gas chromatography is given by Littlewood.[76] A relationship exists between the partition coefficient K and the specific retention volume as shown in equation 15:[77]

$$K = \frac{Vg\rho^{st\ ph}\ T}{273.66} \qquad (15)$$

where Vg is the specific retention volume, T the column temperature, and ρ is the stationary phase density. Similar relationships exist between the enthalpy of dissolution and Vg and the partial molar free energy of solution.

The correlation of retention index with temperature and carbon number has also been considered by Novak.[78] In general, Saura-Calixto[79] found that the plots were linear, and that the effect of temperature was minimal when compared to the effect of carbon number under isothermal conditions.

The relationship between retention index and boiling point was first examined by Kovats[44] who described the differences in the boiling points of two isomers (Δt_b) and their retention indices (dI) on a non-polar stationary phase by the equation:

$$dI \approx 5\Delta t_b \tag{16}$$

or more generally:

$$\Delta I_R \approx \alpha \Delta t_b \tag{17}$$

where α is a constant.

The relationship shown in equation 17 was reviewed by Budahegyi et al.[52] Saura-Calixto and co-workers[80] discussed the correlation between retention indices and boiling points for alcohols, aldehydes, ketones and esters and studied the variation in the value of M in equation 18:

$$I_R = MT_B + C \tag{18}$$

where T_B is the solute boiling point and M and C are constants. Bermejo and Guillen[81,82] related the retention indices of alkanes and chlorobenzenes to the boiling points and molar refraction using multiple regression analysis. The boiling point was considered as the only parameter responsible for retention if the solute-stationary phase interactions were absent, whereas the molar refractivity was chosen to represent those interactions between the solute and stationary phase. This quantity can be related to electronic polarisibility and hence dispersive interactions which are responsible for retention in alkanes.

Lamparcyzk and Radecki[83] related the retention indices to molecular polarisability, ionization potentials and permanent dipole moments whilst Kalizan[84] related the indices to molecular polarity and dipole moments. Significant correlations were found on a single stationary phase between the retention index and electronic parameters but only for those compounds with similar ionization potentials. The retention index of polar compounds was dependent on the dipole moment whereas a linear relationship existed for non-polar molecules between retention indices and polarisabilities. Saura-Calixto and Garcia-Raso[85,86] studied the relationship between van der Waals volumes and retention indices and showed that the relationship:

$$I = aw_v + b \qquad (19)$$

where w_v is the van der Waals volume and a and b are constants, holds for some esters.

Another physico-chemical parameter, the molecular connectivity, has been correlated with retention index. The concept of molecular connectivity was developed by Randic[87] and has found increasing use in

pharmaceutical studies.[88,89] Molecular connectivity describes the size and shape of molecules by means of a topological approach. Each molecular structure is represented as a graph in which each atom is regarded as a vertex and each bond as an edge. If all possible orders of connectivity factors are calculated for a given molecule each factor contributes unique information towards the graphical representation of that molecule. The subject has been extensively reviewed by Kier and Hall[88] who also described the method of calculation.

The molecular connectivity of a given compound has been correlated with such molecular properties as the molar refraction, diamagnetic susceptibility, heat of vaporisation, boiling point, liquid density and water solubility and hence it seemed possible to correlate the molecular connectivity values with retention data such as retention indices. Kalizan[90] correlated the retention indices of alcohols and methyl esters on a non-polar phase with good results but found problems with polar phases. Kalizan and Lamparczyk[91] also found a correlation between the retention indices of polycyclic aromatic hydrocarbons on non-polar stationary phases and the molecular connectivity. Michotte and Massart[92] found a poor correlation between the molecular connectivity indices and the retention volumes of some alcohols, ethers and esters. However Millership and Woolfson[93,94] found that using the logarithm of the retention volume gave improved correlations for these compounds.

Kier and Hall[95] showed molecular connectivity indices could provide a description of structure within a chemical class that was adequate for the correlation of chromatographic behaviour but that chromatographic behaviour across chemical classes, eg alcohols and ketones, depended on non-topological features such as hydrogen bonding,

confirming the earlier work of Michotte and Massart.[92]

Millership and Woolfson,[96] and McGregor[97] showed a relationship between molecular connectivity and retention data (retention index and specific retention volume) for a diverse range of compounds including hydrocarbons, esters, aldehydes, ketones and ethers. Single parameter equations were sufficient to describe non-polar molecules on non-polar phases but for more polar molecules and phases, multi-parameter equations using electronic contributions were required. In these cases poorer correlations were obtained.

Buydens and Massart[98] investigated the correlation between retention indices, connectivity and linear free energy parameters such as the Hammett constant. They found that the topological index was successful for a homologous series whereas a combination of variables was again required for a mixed data set. Subsequently, Buydens, Massart and Geerlings[99,100] related retention indices on stationary phases of different polarities to different structural parameters for bifunctional molecules.

Thus the molecular connectivity approach appears most successful when used for non-polar molecules and stationary phases since the retention is largely dependent on the molecular size and shape. For more polar systems non-topological factors such as dipole moments and local electronic effects are important.

1.4.7 Prediction of Retention Indices from Molecular Structure

There is an increasing interest in the prediction of retention indices from the molecular structure. The methods used for the prediction have been reviewed by Budahegyi et al.[52] Two approaches will be discussed here, the incremental method and topological method.

Kovats[46] studied the factors affecting retention data and proposed rules which formed the basis of retention index predictions.

(a) The retention indices of the higher members of a homologous series increase with the addition of a methylene group by a constant value.

(b) The difference in the boiling points of two isomers is proportional to the difference in retention indices on a non-polar stationary phase.

(c) The retention indices of non-polar compounds on non-polar stationary phases remained constant.

(a) Incremental Method

Takacs and coworkers[101,102,103] developed the incremental method based on the definition of index contributions of chromatographed compounds. The method is dependent on a computerised code system based on the chemical bonds within a molecule, including their primary and secondary environments such that:

$$I_x^{st.ph}(T) = I_a + I_b + I_i^{st.ph} \qquad (20)$$

where I_a is the atomic index contribution, I_b is the bond index contribution and $I_i^{st.ph}$ the interaction index contribution. I_a and I_b are dependent only on the substance analysed and may be calculated from thermodynamic parameters whereas $I_i^{st.ph}$ is also dependent on temperature and stationary phase.

The method has been used by Lombosi[104] for the calculation of alkane retention index with an accuracy of 0.1 units for values between 500-1000 and Fisch[105] calculated the indices of aromatic hydrocarbons in both isothermal and temperature programmed operation. Czerwiec[106]

examined the relationship between molecular structure and retention for chlorobenzenes, chloroanilines and N-sulphonyl anilines and found that the measured and calculated index values were within 4 index units. Other workers using the method include Rang[107,108] for alkynes, Castello and co-workers[109] who identified branched chain alkanes and Morishita[110] for chlorinated alkanes.

This method, however, has serious drawbacks since it is generally only applied to simple molecules or hydrocarbons on non-polar stationary phases. Vanheertum[111] found inaccuracies in the bond and atomic index contributions and suggested the C-H contributions should be differentiated according to the molecular environment. Spivakovskii and coworkers[112] suggested that inaccuracies were due to interactions of the molecules and/or their fragments in dilute solution. These workers also suggested theoretical difficulties with the additivity principle. Since the retention index is a linear function of the Gibbs free energy, it can be represented as a summation of entropy and enthalpy terms. The entropy term depends on the total molecular configuration and this cannot be expressed accurately using additivity principles.

Another possible source of inaccuracy is the lack of universality of the group contributions. The partial interaction energy of a structural fragment depends, not only on the structural fragment itself, but also on the effect of surrounding atoms and groups. These interactions determine the conformational state of the molecule. Thus it is difficult to have universal contributions for structural groups which may be present in different compound classes.

(b) Topological Approach

Chretien and Dubois[113,114,115] used this approach to study alkane chromatographic behaviour and found it necessary to separate the

extensive nature of the index value (the solute characteristics such as ideal thermodynamic behaviour) from the intensive character (related to specific solute-stationary phase interactions). As discussed in section 1.4.6, Randic[87] developed the concept of molecular connectivity index which determines the formal valency of carbon atoms in a molecular graph. It must be emphasised that the molecular connectivity describes the size and volume of the molecule as a whole rather than using individual structural fragments and as such it is an arbitrary, rather than an observable, quantity.

Bonchev and coworkers[116] used a topological approach to distinguish between branched alkylbenzenes based on Wiener Numbers[117,118] which depend on the size and shape of a skeletal network of a given molecule. The retention indices were calculated using the two parameter equation shown below:

$$RI = aW(G)^n \qquad (21)$$

where RI is the retention index, a is a constant and $W(G)^n$ is the Wiener number for that molecule. The maximum error was 15 index units. Paparova and Dimov[119] extended the approach to the use of structural elements and found a maximum error of 8 index units.

Buydens and Massart[98] combined topological and linear free energy parameters to predict retention indices on different polarity stationary phases for alkanes, fatty acid methyl esters and a mixed data set. Again the topological parameters performed best for the homologous series but for the mixed data set it was necessary to include a term describing electronic differences (see references 99, 100). Sablic[120,121] calculated the retention indices of chlorinated alkanes, and benzenes.

Regression analysis showed the approach was successful for both polar and non-polar stationary phases.

The topological approach has advantages in that it requires fewer parameters for calculation than the incremental method. It also helped indicate the structural features responsible for retention, eg for chloroalkanes the size of the alkyl chain was important as well as the topological relationship between the chlorine atoms, whereas for chlorobenzenes the overall size was important.

Doherty and coworkers[122] predicted the retention indices of nitrated polycyclic hydrocarbons and Whalen-Pedersen[123] combined molecular connectivity with 25 other structural parameters to predict the retention indices of polycyclic aromatic hydrocarbons. Rayner, Wiesling and Novotny[124] derived equations to predict retention indices of ketones in temperature programmed operation. The major disadvantage with the approach is that it cannot cope with three dimensional structures.

(c) Alternative Systems for Prediction of Retention Indices

Papazova and Dimov[125] derived a relationship for calculating the retention indices of alkenes, confirming a physico-chemical index, connecting the retentions at different temperatures with pressure and molecular volume, and a structural number expressing the compound's entropy change in a non-polar stationary phase. The disadvantage with the relationship is that it requires a knowledge of the vapour pressure.

Kleinert and Ecknig[126] used two parameters, D and ϕ, where D is a measure of dispersion forces between solute and stationary phase and ϕ is a measure of the energy of association interaction given by the sum of intermolecular interactions caused by polar functional groups.

Gassiot-Matas and Firpo-Pamies[127] used a relationship derived from

electrostatic interactions combined with molecular connectivity, with the solute structure defined using molecular connectivity and electrostatic interactions defined using the dipole moment.

1.4.8 Application of Retention Indices to the Classification of Stationary Phases

In addition to the qualitative analysis of gas chromatographic retention data, retention indices may be used to characterise stationary phase polarity. Rohrschneider[128] proposed a scheme also suitable for the prediction of retention indices, based on the additivity of intermolecular forces which was later extended by McReynolds[129]. The concept was summarised by Budahegyi and coworkers[52] for constant gas-liquid chromatographic conditions. The principle of this scheme is the additivity of intermolecular forces. These are evaluated from the differences in retention index values of a series of test probes measured on the liquid phase to be characterized and squalane, a non-polar phase. The series of test probes must adequately characterize the principle molecular interactions responsible for retention in gas chromatography: dispersion, orientation, induction and donor-acceptor complexation. Rohrschneider[128] suggested that benzene, ethanol, 2-butanone, nitromethane and pyridine should be used and McReynolds[129] extended the list to ten compounds.

McReynolds[129] assumed that for each type of polar intermolecular interaction, the interaction energy is proportional to values a, b...e characteristic of each test probe and values x', y'...s' characteristic of the liquid phase. The retention index difference ΔI is thus compiled from products as shown in equation 22 below:

$$\Delta I = ax' + by' + cz' + du' + es' \qquad (22)$$

The liquid phase constants x', y'... s' are determined by injecting each of the test probes and hydrocarbon retention index standards on the phase to be characterized. The phase specific constants are calculated by subtracting the retention index on squalene from that measured as the phase to be characterized as shown in equation 23 below:

$$x' = \Delta I = I^{phase} - I^{squalene} \qquad (23)$$

Where x' is a phase specific constant and I is the change in retention index, I^{phase} and $I^{squalene}$ are the retention indices on the given phase and squalene respectively. Harkopf[130,131] has extended this approach to the use of four functional probes with the same accuracy as the Rohrschneider constants.

1.5 WALL COATED OPEN TUBULAR COLUMNS AND RETENTION INDICES IN ENVIRONMENTAL ANALYSIS

1.5.1 WCOT Columns in Environmental Analysis

With the development of open tubular columns and associated techniques such as linear temperature programming in gas chromatography, it has become possible to analyse increasingly complex samples with wide ranges of polarities and boiling points.

Organic trace analysis of environmental samples deals with complex mixtures from a wide variety of sample matrices from different locations. They may have undergone biotic or abiotic degradation and may thus be multi-component and multimatrix requiring a strategy to reduce the overall complexity before the gas chromatographic separation. In this discussion the emphasis will be on organochlorine (OCs) pesticide and polychlorinated biphenyl (PCBs) analysis.

Prior to the use of WCOT columns in the analysis of PCB mixtures, most procedures attempted to measure the whole class without providing complete separation of individual components. A comparison of packed and WCOT column separation was performed by Grob[132] on a lake water extract using the same stationary phase. Although the WCOT column was 12 times longer it had 1% of the stationary phase but performed a more efficient separation in a shorter time. Comparisons of the two column types were also done by Kominar and co-workers[133] and Zenon-Roland and co-workers[28] with the open tubular columns having superior sensitivity and resolution over the complete range and improved reproducibility. Krupcik and coworkers[134] found that unlike a glass open tubular column, packed columns were incapable of separating a PCB mixture under isothermal conditions.

Poy[135] discussed environmental analysis using WCOT columns and

suggested that good sampling and system techniques were crucial for high-efficiency separation and reproducibility and accurate quantitative results in small sample sizes. Sauter, Betowski et al[136] used fused silica columns for priority pollutant analysis and these columns allowed the analysis of both acid and base extractions which were not possible with packed columns. Qualitative analysis was performed using relative retention times with multiple internal standards. The column was also more tolerant of complex samples but some problems were encountered with column overload.

Albro, Corbett and Schroeder[137] used glass columns to analyse PCB mixtures and stated that their retention behaviour was sufficiently different on different liquid phases that it would be possible to find a phase capable of resolving any given PCB pair. Thus the possibility of double assignment would be removed by the use of two phases. In general, medium polarity phases were best because PCBs have a low solubility on high polarity phases and low polarity phases give insufficient separation.

Dahlgram and Adam[138] found WCOT columns were the only suitable columns for the resolution and quantitation of priority pollutant acid extractables. The columns were acid and/or base conditioned and the largest performance change was noted when a single column was used for base/neutral extractables followed by acid extractables with evidence of peak tailing and skewing.

Colby and coworkers[139] found high degrees of precision and excellent inter-laboratory agreement for the analysis of 28 test compounds using fused silica columns.

1.5.2 Retention Indices in Environmental Analysis

Ballschmiter[140] discussed in detail the concept of high resolution gas chromatography in environmental analysis. He argued that the simplified picture shown in the early 1970s resulted from the techniques used rather than the nature of the samples investigated and the use of WCOT columns creates a much more detailed image. However, the drawback with this method was the great volume of data generated.

The basis of any identification is either the comparison of retention data of unknowns with that of reference compounds or the use of mass spectrometers. The former method required that the data must be standardised independently of the equipment, operator and conditions of analysis. The recommended reference system was the Kovats' retention index system and since it is more usual to use temperature programming, retention indices are only formally equivalent to the isothermal Kovats' indices.

Sissons and Welti[141] compared the use of retention indices with that of a standard addition method for PCB identification. In this system, the retention index increment for a defined substitution pattern is summed for a new substitution pattern. The method was found to be a highly accurate and reproducible technique. Betty and Karasek[142] combined GC-MS with retention indices for compound identification since mass-spectrometry data are not always sufficient to identify closely related compounds such as isomers. They found Kovats' indices provided a relatively invariant data set.

Neu and Zinburg[143] discussed the concept of retention indices and the use of high resolution gas chromatography. They suggested that proving the reliability of compound identification is possible in two ways; either by running the sample on two columns of different polarity or

by increasing the separation efficiency. The authors postulated that a single high resolution chromatogram could give sufficient information for a qualitative evaluation of composition provided the retention data were measured with sufficient accuracy. It is possible the retention index system could give sufficient accuracy and the method is not restricted to the use of n-alkanes as reference compounds (see section 1.6).

1.5.3 Development and Use of Internal Standards in Environmental Analysis

With the development of open tubular columns, increasing use has been made of gas chromatography in the determination of contaminants in environmental samples. The use of these high efficiency columns, with the inherently small (0.1-2µl) injection volumes and the long analysis times (over 40min) for multi-component analysis has made the use of internal standards for accurate quantitation almost essential.

There are few internal standards commonly used in quantitative environment analysis at present whereas considerable use is made of internal standards in medical science. Wells and Cowan[144] reported that, in the period 1980-1982,[145] 73% of the applications in the GC-MS Abstracts Clinical Chemistry section used internal standards whereas in environmental science only 10% of the abstracted papers used internal standards.

Those internal standards which have been used in both environmental and medical science were generally tailor made for their applications such as deuterated polyaromatic hydrocarbon isotopic homologues, ^{37}Cl labelled isomers of tetrachloro dibenzo-p-dioxins and ^{14}C and ^{18}O labelled compounds in many drug metabolite studies and the tracing of biosynthetic pathways. These standards have the advantage that they may be added to a

sample prior to extraction, 'clean-up' or derivatisation procedures and undergo similar modifications to the unlabelled moiety. However, the extensive use of labelled compounds as internal standards has a number of drawbacks. The most important of these is that their use is restricted to GC-MS which is an expensive and relatively complex technique. They are also only suitable for the analysis of specific compounds or mixtures containing relatively few compounds. Their preparation and testing for each determination is expensive and time consuming and for multi-component analysis, as is usually the case in environmental analysis, this is not a feasible proposition on economic or logistical grounds. Those internal standards which have been used in environmental analysis include Aldrin, tetrachloronaphthalene, Mirex and decachlorobiphenyl[144] but these also suffer from drawbacks including possible environmental contamination. There is thus a requirement for a compound or a group of compounds which may be used as an internal standard or standards in complex multi-component analyses.

1.5.4 Use of Internal Standards in Qualitative and Quantitative Analysis

The use of internal standards has been generally associated with the quantitation of sample peaks (see section 1.2.4). However, their use may be extended to the identification of peaks. Since retention times may be stated relative to an internal standard or standards, in this context the internal standard serves the same purpose in both quantitative and qualitative analysis, allowing the normalisation of raw data independent of the equipment, time and place of analysis and operator. Thus the inclusion of an internal standard in the sample allows the measurement of retention data whether peak height and area, or retention time, as a

relative rather than an absolute value.[50]

This feature was particularly important where packed columns were employed but less so for open tubular columns in which the 'column dead time' plays a much less significant role. However, the use of internal standards is still important for the determination of any systematic errors which may be inherent in the system and makes increased accuracy and precision in both quantitative and qualitative analysis possible.

Most gas chromatographic analyses reported up to 1979 employing an internal standard used a single compound to which the time and response of all eluants in the chromatogram could be related. This approach was regarded as sufficiently accurate for most isothermal analysis on packed columns and with short analysis times (up to 20 min). However, with the introduction and routine use of open tubular columns, longer analysis times and complex, multi-component samples have been increasingly encountered and one internal standard has proved insufficiently accurate. Sauter and coworkers[136] reported that in the analysis of priority pollutants one internal standard did not cover the entire chromatogram with similar precision and the precision was lowest for compounds which eluted furthest from the internal standard. These findings were confirmed by Colby, Ryan and Wilkinson[139] who found that the retention times of the sample peaks showed a marked increase in precision for those relative retention times close to 1. Furthermore they demonstrated that retention time 'windows' ($\pm$3 standard deviation windows expressed in seconds) increased in both directions from the internal standard retention time and become equivalent to absolute retention time 'windows' at either end of the chromatogram. The use of multiple internal standards allowed precision close to that for isotopic internal standards over the long term widths at both ends of the chromatogram. The results also indicated that accurate quantitation could

best be obtained by use of isotope dilution but that an internal standard approach was approximately twice as accurate as the external standard approach in the long term. The authors suggested that internal standard selection was essential for effective identification and quantitation of compounds. For reliable identification, the most closely eluted internal standard was recommended whereas the most reliable quantitation was obtained using a chemically 'similar' standard regardless of elution time. Wells and Cowan[144] also confirmed the results of Sauter et al and found good reproducible results with an accuracy of ±1% could be obtained for qualitative analysis using multiple internal standards. As with the retention times, the coefficient of variation on relative peak areas increased the greater the time separation between analyte and internal standard. Thus the precision in peak retention times and areas were improved by the use of multiple internal standards which eluted within a ±10 minute time interval.

Thus the use of multiple internal standards spaced throughout the chromatogram could improve the accuracy of the relative retention times so that it is close to that obtained with the use of isotopically labelled samples. The development and use of a series of compounds could improve the measurement of gas chromatographic retention data over that obtained with single standards especially for the complex mixtures encountered in environmental analysis, so that both the accuracy and precision of quantitative work and also the reliability of peak identification is increased markedly.

An alternative to multiple internal standards would be a retention index system similar to those discussed in 1.5.2. To be useful in environmental analysis they must fulfil certain criteria, as described below.

1.6 COMPOUNDS SUITABLE FOR USE AS RETENTION INDEX MARKERS AND INTERNAL STANDARDS

1.6.1 Criteria for Suitability of Compounds

The most widely used concept is that of the Kovats' retention index system. The original primary standards employed were the n-alkanes. However, these compounds are unsuitable for use in many environmental analyses in which an electron capture detector is used since they have little response or a negative response with this detector. Thus an alternative series of compounds is required which can be used with an electron capture detector and preferably with other commonly used detectors such as flame ionization detector and mass spectrometer. Such a series would be advantageous also if the members could be used not only as retention index calibration standards but also as multiple internal standards for the quantitation of samples under analysis. The criteria for such a series are described below:

a. The homologous series should be easily prepared and purified.

b. The compounds should be thermally and photochemically stable and have a 'reasonable' shelf life of at least two years.

c. The compounds should be non-toxic and easy to handle.

d. They should not be present in any environmental sample under consideration.

e. The compounds should be suitable for use in both gas and liquid chromatography.

f. The compounds should be suitable for use with a range of detectors, in particular the electron capture detector, flame ionization detector, mass spectrometer and, for liquid chromatography, the ultra-violet spectrophotometer.

g. The series should possess a wide range of retention times (0-4000 sec) and elution temperatures (80-300°C) and each member of the series should be resolved from any compound of interest in the chromatogram.

h. They should also be suitable for use with linear temperature programming in chromatography.

Alternative compounds have been considered for this purpose. Nakamura and coworkers[146] used chlorobenzenes and decachlorobiphenyl which are useful as internal standards but since they are not a homologous series are unsuitable for use as retention index markers. Kozloski[147] used polychlorinated biphenyls obtained by partial dechlorination of individual PCBs with up to six chlorine atoms as retention standards and the compounds were co-eluted with n-alkanes to allow the calculation of retention indices. The principle drawbacks with the method are that it is largely restricted to the PCB analysis, identification of the individual congenors could only be achieved after extensive cross-checking of reported retention index values and cross-comparison with peaks generated from the different parent PCBs. Thus the preparation of any dechlorination mixture would first have to undergo extensive testing before use. The advantage with the method for PCB analysis is that the retention standards and compounds of interest would be very similar chemically and the chromatographic behaviour of both should be similar with the resultant benefits for quantitation.

Pacholec and Poole[148,149] employed n-bromoalkanes as retention index markers for the calibration of open tubular column gas chromatography with electron capture detection. These compounds have the advantage that they are part of a homologous series and one which is fairly closely related to the n-alkanes. In chemical terms they are

relatively simple with the presence of the bromine atom being the only functionality; thus the steric effects inherent in the system again would be similar to those present with the n-alkanes. The authors employed the series pentyl to octadecyl bromo-alkanes and found the compounds suitable for use in linear temperature programming with temperatures up to 200°C and the standard deviation of retention indices was 0.35 retention index units (RIU). One of the major drawbacks of the series is that higher molecular weight n-bromoalkanes would be required to cover the volatility range encountered in environmental samples since the upper limit of retention index values was approximately 1500 RIU and the upper elution temperature was 200°C and it must also be noted that the retention indices were not calculated with direct reference to the n-alkanes but instead the compounds were assigned empirical values. Other drawbacks of the n-bromoalkanes include the lack of a characteristic fragment with m/z over 100 in the mass spectral fragmentation pattern, and the spectrum is dominated by the alkyl system. There may also be the possibility of n-bromoalkanes occurring in environmental samples.

Ballschmiter and coworkers[150] developed the n-alkyl trichloroacetates for use as retention index markers in PCB and organochlorine analysis and their preparation was described by Schwartz.[151] Again these compounds are sensitive to electron capture detection but do not possess a characteristic mass spectral fragment. Also, esters are readily susceptible to acid and base hydrolysis and hence could not be added to samples before 'clean-up' procedures. Esters are also susceptible to poor chromatographic performance as a result of their sensitivity to any active sites.

1.6.2 Choice of Compounds for use as Retention Index Markers and Internal Standards

To fulfil the criteria described in section 1.6.1 the series of compounds chosen must possess certain structural characteristics. Compounds readily detected by the ECD should be halogenated with four or more fluorine atoms, two or more chlorine atoms or one or more bromine atoms, whereas those for use with FID should possess a hydrocarbon chain. Ideally for mass spectrometry, there should be a characteristic mass fragment between m/z 100 and m/z 200 for convenience of use in single ion monitoring. The fragment should also not coincide with any background interference resulting from compounds such as phthalates etc. For ultraviolet detection in liquid chromatography the compounds should have an aromatic system present.

Compounds which were considered to fulfil the above criteria were the pentafluorobenzyl alkyl ethers or esters, the polychloronaphthyl ethers and esters, the polyfluorobenzyl alkyl ethers or esters and the chlorinated benzyl alkyl ethers or esters. Ethers were chosen in preference to the esters because of the possibility of ester hydrolysis and the susceptibility of esters to poor chromatographic performance. Chlorinated rather than fluorinated systems were chosen because of their general ease of preparation compared with the fluoro systems.

The series chosen for examination was the 2,4-dichlorobenzyl alkyl ethers since they should be relatively unreactive with well documented methods of preparation from readily available starting materials. The benzyl system was chosen in preference to the naphthyl system because it was felt it would cover the desired chromatographic range more readily.

CHAPTER TWO

MATERIALS AND METHODS

2.1 REAGENTS AND INSTRUMENTATION

2.1.1 Preparation and Purification of 2,4-Dichlorobenzyl Alkyl Ethers

The starting materials 2,4-dichlorobenzoic acid, 2,4-dichlorobenzyl alcohol, n-bromoalkanes, sodium hydride and potassium tert-butoxide were obtained from the Aldrich Chemical Company Limited (Gillingham, Dorset, England) with the exception of 1-bromoundecane and 1-bromotridecane which were obtained from Koch Light Ltd (Haverhill, Suffolk, England).

All materials were obtained as GPR grade.

The solvents used were either obtained as HPLC grade from the Rathburn Chemical Company Limited (Walkerburn, Scotland), or as reagent grade from May and Baker (Dagenham, England) and purified before use. Diethyl ether and petroleum ether were purified by distillation from calcium hydride, dimethyl formamide by distillation from powdered calcium hydride under reduced pressure and ethyl acetate by distillation from phosphorus pentoxide.

Thin layer chromatograms were recorded using Merck (5735) plates (BDH Chemicals, Thornliebank, Glasgow, Scotland) and an elution scheme of 90:10 petroleum ether:EtOAc or hexane:EtOAc.

The 'Chromatotron' was supplied by TC Research, St Albans, Hertfordshire, England.

Short silica columns were made using silica Kieselgel 7730 (BDH Chemicals Limited, Thornliebank, Glasgow, Scotland) and an elution scheme of 99% hexane or petroleum ether /1% ethyl acetate.

NMR spectra were recorded using a Perkin-Elmer R-24 60MHz spectrometer and a Perkin-Elmer R32 90MHz spectrometer.

IR spectra were recorded using a Perkin-Elmer 577 Grating Infra-Red spectrophotometer.

Mass spectra were recorded using a Jeol JMS D100 high resolution mass spectrometer and GC-mass spectra were recorded using a Finigan 3200F GC-MS Electron ionization 25eV, and/or 70eV, multiplier 1700V and fitted with an SGE OCI-2 on-column injection system. The carrier gas was helium at a flow-rate of 27cm sec^{-1} and a CPSil5-CB column from Chrompack UK Limited (Millharbour, London, England).

2.1.2 Analysis of 2,4-Dichlorobenzyl Alkyl Ethers by Gas Chromatography

Gas chromatograms were recorded using a Varian 3700 gas chromatograph fitted with an electron capture detector (63-Ni) with a detector temperature of 350°C and an on-column injection system (modified SGE-OCI3) and a Pye 304 gas chromatograph with a flame ionization detector and an electron capture detector (at 350°C). A splitless injector was used at 275°C. The carrier gas for both GCs was hydrogen with a linear velocity of 35-37cm of column sec^{-1}. The columns used were as stated in the methods and were supplied by Chrompack UK Ltd (Millharbour, London, England) with dimensions of 25m x 0.22mm.

The data was acquired using an Apple IIe microcomputer with an Adalab A/D interface and running the 'Chromatochart' programme (Heyden and Sons).

Data from the GC-MS was acquired using a Finigan 6110 Data System.

2.1.3 Gel Permeation Chromatography

The column used was 25mm internal diameter and 30cm length packed with SX-3 bio-beads (BioRad, Watford, England).

The pump was an Altex 100A and the detector was a Cecil 212 variable wavelength UV detector.

2.2 PREPARATION OF 2,4-DICHLOROBENZYL ALCOHOL

2.2.1 Attempted Reduction of 2,4-Dichlorobenzoic Acid

Lithium aluminium hydride ($LiAlH_4$) (5g) was suspended in dry diethyl ether (50ml) under nitrogen and stirred. 2,4-Dichlorobenzoic acid (5g) in dry diethyl ether/tetrahydrofuran (THF) (80/20 v/v) was added dropwise at such a rate as to maintain reflux. After the addition was complete the mixture was refluxed for one hour. The reaction was cooled to room temperature and a solution of saturated ammonium chloride in water (25ml) added to destroy any unreacted $LiAlH_4$. The mixture was filtered, the filtrate washed with a saturated sodium carbonate solution and extracted with 3 x 25ml aliquots diethyl ether. The combined extracts were dried over anhydrous magnesium sulphate and the magnesium sulphate removed by filtration. The solvent was removed using a rotary evaporator leaving an orange oil (3g) which was dissolved in carbon tetrachloride and reconcentrated. This procedure was repeated twice. On leaving overnight, the residue solidified giving orange crystals which were dried overnight in a vacuum desiccator. An infra-red spectrum indicated the presence of unreacted acid (carbonyl stretch 1785cm^{-1}) so the material was recycled using a further amount (2g) of $LiAlH_4$ and refluxing overnight. The work-up procedure was as before.

Infra-red spectra 3600cm^{-1}, 3400cm^{-1} C-OH stretch 2900 C-H stretch 1470cm^{-1} aromatic C=C stretch .

2.2.2 Attempted Large Scale Reduction 2,4-Dichlorobenzoic Acid

Dichlorobenzoic acid (25g) in dry THF (150ml) was added dropwise to

a suspension of $LiAlH_4$ (10g) in dry THF (350ml) at such a rate as to maintain reflux, after which the reaction was refluxed overnight.

The reaction mixture was cooled and ethyl acetate (100ml) added followed by dilute HCl (1M, 50ml). The mixture was filtered, extracted with diethyl ether and dried as previously described. An infra-red spectrum indicated the presence of unreacted acid and the reaction was repeated using the above product. After the repetition the infra-red spectrum indicated the acid was still not fully reduced.

Infra red spectrum carbonyl stretch at $1750cm^{-1}$.

2.3 PREPARATION OF DICHLOROBENZYL ALKYL ETHERS

2.3.1(a) 2,4-Dichlorobenzyl Octadecyl Ether, THF as Solvent.

Using the product of 2.2.1, 0.91g, was dissolved in dry THF (15ml) and added to a suspension of sodium hydride (0.11g) in dry THF (20ml) and the mixture stirred for one hour. 1-Bromooctadecane (1.8g) in dry THF (15ml) was added and the reaction stirred at room temperature overnight.

A saturated solution of ammonium chloride (25ml) was added and the aqueous phase extracted with diethyl ether (3x25ml aliquots). The combined extracts were dried over magnesium sulphate, filtered and the solvent removed. The product was a yellow oil (3g) and a TLC indicated the presence of unreacted alcohol.

An infra-red spectrum showed a hydroxyl stretch at $3200cm^{-1}$.

2.3.1(b) Dimethylformamide as Solvent

A solution of 2,4-dichlorobenzyl alcohol (2g) in dry dimethylformamide (DMF, 20ml) was added dropwise to a suspension of dry sodium hydride (0.2g) in dry DMF (15ml) and the mixture stirred for 1

hour at room temperature. 1-Bromooctadecane (3.7g) in dry DMF (10ml) was added and the reaction stirred overnight at 75°C. The DMF was removed on a rotary evaporator and the residue dissolved in water (100ml). The solution was extracted with diethyl ether (4x25ml aliquots) and the combined extracts dried over magnesium sulphate. The mixture was filtered and the solvent removed. The product, an orange solid, was recrystallised from hexane giving white crystals, melting point 45-48°C. The yield was approximately 3g.

Infra-red spectrum C-H stretch at 2900, ether stretch 1475cm^{-1}.

For nmr data, please see Table 2.1A.

2.3.2 Tetradecyl, Dodecyl, Decyl Ethers

The above method was repeated in each case using 2g 2,4-dichlorobenzyl alcohol to give the corresponding products in satisfactory yields (40-60%). The spectral characteristics of the products are summarised in Tables 2.1A and 2.1B.

Table 2.1A NMR Spectra of Tetradecyl, Dodecyl and Decyl Ethers.

Ether	Aromatic Multiplet (3H)	Benzylic CH_2 Singlet (2H)	Non-Benzylic (CH_2) Triplet (2H)	$C_n H_{2n+1}$ Multiplet
Tetradecyl n=13	7.2	4.4	3.5	0.8 - 1.5
Dodecyl n=11	7.2	4.7 (multiplet)	3.4	0.7 - 1.1
Decyl n=9	7.2	4.5	3.3	0.7 - 0.9

All spectra recorded at 60 MHz.
Solvent CCl_4.

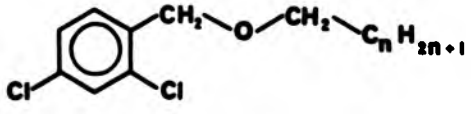

Table 2.1B IR spectra of tetradecyl, dodecyl and decyl ethers.

Ether	Aliphatic and Aromatic C-H stretch cm^{-1}	Aromatic C=C stretch cm^{-1}		Ether C-O-C stretch cm^{-1}
Tetradecyl	2900 - 3000	1595	1475	1100
Dodecyl	2800 - 3000	1575	1465	1095
Decyl	2800 - 2900	1570	1479	1100

2.4. PREPARATION OF 2,4-DICHLOROBENZYL ALKYL ETHERS - GENERAL METHODS

2.4.1 Butyl(C_4), Hexyl(C_6), Heptyl(C_7), Octyl (C_8), Nonyl(C_9), Decyl(C_{10}), Undecyl(C_{11}), Dodecyl(C_{12}), Tetradecyl(C_{14}), Hexadecyl(C_{16}) Ethers.

These compounds were prepared using commercially available 2,4-dichlorobenzyl alcohol. The method used is described in detail for the hexyl ether. The quantities of reagents used for the preparation of the remaining ethers were similar.

2,4-Dichlorobenzyl alcohol (2g) was dissolved in dry DMF (20ml) and added dropwise to a suspension of dry sodium hydride (0.3g) in dry DMF (15ml) and the mixture stirred for 1 hour at room temperature. A solution of 1-bromohexane (1.86g) in DMF (10ml) was added and the reaction stirred at 60°C overnight.

After cooling, the DMF was removed on a rotary evaporator, the residue dissolved in water (50ml) and extracted with diethyl ether (4x25ml). The combined extracts were dried over magnesium sulphate, filtered and the solvent removed to give the product in satisfactory yield (30-60%).

The ethyl ether was prepared using 1-iodoethane instead of the bromo-alkane. The infra-red and nmr spectral details are summarised in Tables 2.2 and 2.3.

Table 2.2 Principle bands in IR spectra of 2,4-dichlorobenzyl alkyl ethers.

	Peak Frequencies cm^{-1}			
	Aliphatic and Aromatic C-H Stretch	Aromatic C=C Stretch		C-O Ether Stretch
Ethyl	2900-3000	1600	1470	1100-1150
Propyl	2900-3000	1595	1475	1100-1150
Butyl	2850-3000	1570	1470	1100-1150
Pentyl	2850-3000	1600	1475	1100-1150
Hexyl	2700-3020	1570	1475	1100-1150
Heptyl	2800-2900	1580	1475	1100-1125
Octyl	2850-2950	1570	1475	1100-1150
Nonyl	2850-2950	1580	1470	1100-1125
Decyl	2850-3000	1600	1470	1100-1150
Undecyl	2850-3000	1600	1475	1100-1150
Dodecyl	2800-3000	1600	1470	1100-1150
Tridecyl	2850-3000	1595	1475	1100-1150
Tetradecyl	2800-2950	1600	1475	1100-1150
Pentadecyl	2800-2950	1570	1465	1100-1150
Hexadecyl	2900-3000	1595	1475	1100-1150

Table 2.3 Principle peaks in NMR Spectra of 2,4-Dichlorobenzyl Alkyl Ethers.

	Principle Peaks in NMR Spectra			
	Aromatic Multiplet (3H)	Benzylic CH_2 Singlet (2H)	Non Benzylic CH_2 Triplet (2H)	$C_n H_{2n+1}$ Multiplet
Ethyl	7.00-7.50	4.50	3.50(quartet 2H)	1.30(triplet 3H
Propyl	7.00-7.50	4.45	3.45-3.55	0.70-1.30
Butyl	7.00-7.40	4.40	3.40-3.70	0.80-2.05
Pentyl	7.05-7.40	4.45	3.35-3.55	0.85-1.80
Hexyl	7.15-7.50	4.45	3.35-3.55	0.80-1.80
Heptyl	7.00-7.50	4.55	3.35-3.55	0.75-1.80
Octyl	7.00-7.40	4.45	3.35-3.65	0.80-1.80
Nonyl	7.00-7.50	4.55	3.45-3.70	0.80-1.90
Decyl	7.15-7.45	4.45	3.30-3.65	0.50-2.00
Undecyl	7.15-7.50	4.55	3.45-3.65	0.75-1.85
Dodecyl	7.10-7.55	4.45	3.35-3.55	0.45-2.00
Tridecyl	7.10-7.50	4.50	3.40-3.80	0.60-2.00
Tetradecyl	7.20-7.40	4.45	3.35-3.65	0.50-1.85
Pentadecyl*	7.15-7.50	4.50	3.45-3.65	0.50-1.85
Hexadecyl	7.10-7.40	4.45	3.35-3.70	0.50-2.00

All spectra recorded at 60 MHz except * - recorded at 90 MHz.

Solvent - CCl_4.

2.4.2 Propyl(C_3), Pentyl(C_5), Tridecyl(C_{13}), Pentadecyl(C_{15}) Ethers

These compounds were prepared using a similar procedure described in detail for the tridecyl ether. The quantities of reagents used for the remaining compounds were similar.

Potassium tert-butoxide (1.4g) was dissolved in DMF (15ml) and 2,4-dichlorobenzyl alcohol (2g) in DMF (10ml) added. The mixture was stirred at room temperature for 1 hour after which 1-bromotridecane (3g) in DMF (10ml) was added dropwise and the reaction stirred at room temperature for a further two hours.

The reaction was quenched with water (50ml) followed by dilute HCl (10ml, 1M) and the mixture extracted with diethyl ether as before. The extracts were dried over magnesium sulphate, filtered and the solvent removed to give products in satisfactory yields of 30-70%. Nmr and infra-red spectral details of the products are given in Tables 2.2 and 2.3.

2.4.3 Ethyl-Octadecyl Ethers - General Method of Preparation

A further batch of ethers was prepared using a modified method from section 2.4.2. The procedure is described for the dodecyl ether and the quantities used for the other compounds were similar.

2,4-Dichlorobenzyl alcohol (5g) in DMF (15ml) was added to a solution of potassium tert-butoxide (3.48g) in DMF (20ml). The reaction was stirred for 30 minutes at $0^{o}C$, then a solution of 1-bromododecane (7.03g) in DMF (10ml) was added and the reaction stirred for 2 hours. The reaction was quenched with water (20ml) and 1M HCl (10ml) and extracted as previously described to give products in satisfactory yields of 30-75%.

2.5 PURIFICATION OF 2,4-DICHLOROBENZYL ALKYL ETHERS

2.5.1 Preparation of Rotor Plate for 'Chromatotron'

A rotor plate with a 4mm thick absorbent layer was prepared. A glass rotor plate was cleaned with detergent, rinsed, dried and mounted on a coating arbor. Masking tape was attached around the edge to prevent leakage.

A slurry of $SiO_2/CaSO_4.1/2\ H_2O$ (100g) in ice cold water (200ml) was prepared and the mixture shaken well. The slurry was poured onto the edge of the plate while it was slowly turned and air bubble formation was prevented by gently tapping the plate on the bench. The rotor was covered and the absorbent allowed to set for 25 minutes after which it was dried overnight at 70°C. The rotor was cooled and the absorbent scraped to the required depth, removing any loose silica from the surface.

2.5.2 Purification of Tetradecyl, Dodecyl and Decyl Ethers

The procedure used is described in detail for the tetradecyl ether, the preparation of which is described in 2.3.2. Polar impurities were removed from the sample by filtering through a sintered glass funnel containing a 2mm layer of silica gel using ethyl acetate as the solvent. The rotor plate was first saturated with solvent (94.5% hexane/5% ethyl acetate/0.5% methanol) under a stream of nitrogen and the system allowed to equilibrate for 5 minutes. The tetradecyl ether sample (1.5g) was dissolved in hexane (2ml) and pumped onto the plate at 4ml min^{-1} and eluted using a flow rate of 2ml min^{-1}. The separation was followed by ultra-violet light and two major fractions were collected (R_f 0.4, 0.6-0.8). The plate was cleaned by eluting with ethyl

acetate to remove polar impurities.

A TLC of the fraction R_F 0.6-0.8 showed two spots and the separation was repeated using hexane as the eluant. Two distinct components were separated. The method was repeated for the dodecyl and decyl ethers.

2.5.3 Ethyl, Butyl, Hexyl, Octyl, Decyl, Dococyl, Tetradecyl Ethers

The 'Chromatotron' was used to purify the above compounds, (cf 2.4.1 and 2.4.2 for details of compound preparation) and the procedure is described in detail for the tetradecyl and dodecyl ethers. In each case polar impurities were removed by purification on a short column containing silica gel (20g) and using elution conditions of 90% petroleum ether (40-60)/10% ethyl acetate, resulting in a mixture of 3 compounds R_F 0.3-0.5.

(a) Tetradecyl ether - initially hexane was chosen as the eluant but did not give satisfactory separation. A modified 3 component mixture of 97.5% hexane/2% ethyl acetate/0.5% methanol gave separation of two fractions (R_F 0.48, 0.33). The nmr spectrum confirmed the presence of the tetradecyl ether in the fraction R_F 0.48. The nmr spectral details are given in Table 2.3.

(b) Dodecyl ether - the elution conditions chosen were 97.5% hexane/2% ethyl acetate/0.5% methanol. The sample was loaded using a 'dry loading' technique.

The absorbent was saturated with hexane to remove impurities and then allowed to dry in a stream of nitrogen. The dodecyl ether (1g in 2ml hexane) was loaded through the pump and allowed to dry in a narrow band on the plate prior to eluting with solvent. The separation was then completed as described in 2.5.3(a). This method was repeated for the rest of the compounds listed.

2.5.4 Purification of Heptyl, Nonyl and Undecyl Ethers using a Silica Column

The column was packed using 20g silica gel for each 1g of sample. The procedure is described below for the nonyl ether.

Silica gel (60g) was mixed as a slurry with the eluant (98.5% petroleum ether (40-60)/1% ethyl acetate/0.5% methanol (200ml)) and poured into a column with a sintered glass base (internal diameter 4cm, length 30cm). The slurry was stirred to minimise air bubble formation and the solvent pumped through using compressed air. The solvent was collected and recycled through the column until the silica bed had settled leaving a layer of solvent (1mm) at the top of the column. The reaction residue (3g) was dissolved in the eluant (2ml) and applied as evenly as possible onto the column head using air pressure. The solvent was eluted through the column using compressed air. Fractions (15ml) were collected and the separation followed by TLC. The nonyl ether was present in the fraction eluted at R_F 0.45. The details of the IR spectra are given in Table 2.2 and the nmr spectra in Table 2.3.

2.5.5 2,4-Dichlorobenzyl Alkyl Ethers from Section 2.4.3

These were purified by the column chromatography method described in 2.5.4 using 1% ethyl acetate/99% hexane as the eluant.

2.5.6 Distillation and Sublimation of 2,4-Dichlorobenzyl Alkyl Ethers

The final purification step was distillation of the liquid phases under reduced pressure using Kugelrühr apparatus. The solid materials were sublimed under reduced pressure. The conditions are summarised in Table 2.4.

Table 2.4 Yields of 2,4-dichlorobenzyl alkyl ethers and conditions used for purification.

	Crude Yield %	Yield Pure Ether g	Temp of Dist. or subl. °C	Pressure mmHg
Ethyl	30%	2.5	50	0.3
Propyl	34%	3.7	70	0.3
Butyl	70%	5.3	110	0.2
Pentyl	40%	4.3	90	0.5
Hexyl	75%	6.5	130	0.3
Heptyl	70%	6.5	150	0.3
Octyl	41%	5.5	155	0.3
Nonyl	40%	2.8	145	0.2
Decyl	46%	4.0	180	0.3
Undecyl	52%	4.8	175	0.5
Dodecyl	47%	4.9	75*	0.3
Tridecyl	56%	6.0	198*	0.5
Tetradecyl	48%	5.6	80*	0.4
Pentadecyl	35%	3.1	95*	0.5
Hexadecyl	36%	4.4	65*	0.1

* Sublimed.

2.6. STABILITY OF 2,4-DICHLOROBENZYL ALKYL ETHERS (DCBEs) TO METHODS FOR CHEMICAL CLEAN-UP OF LIQUID EXTRACTS

A composite mixture of the 2,4-dichlorobenzyl alkyl ethers (0.1 mgl^{-1}) with tetrachloronaphthalene (0.01 mgl^{-1}) as an internal standard in iso-octane was prepared.

2.6.1 Concentrated H_2SO_4

The DCBE solution (1ml) was shaken in an automatic shaker (5 min) with H_2SO_4 (3 drops). The mixture was extracted with hexane-washed water (10ml) and the organic phase examined by GC.

2.6.2 Alcoholic KOH

The DCBE solution (1ml) was concentrated to dryness and saturated alcoholic KOH (0.5ml) added. The solution was heated in a water bath at 60°C for 15 minutes after which it was extracted with hexane (10ml) and the hexane fraction examined by GC.

2.6.3 Methyl Iodide

0.05M Tetrabutylammonium hydroxide/5M ammonium hydroxide (0.5ml) and methyl iodide (100µl) were added to the DCBE mix (1ml). The mixture was shaken for 30 minutes then it was diluted with hexane-washed water (9ml). 15% CH_3COOH/hexane (1ml) was added and the mixture shaken for 1 minute. The organic layer was separated and the solvent removed. The residue was re-dissolved in iso-octane and examined by GC.

2.6.4 Alumina Column

A column containing acid (2g) and basic (1g) alumina was prepared

using the method of Wells and Johnstone.[152] The DCBE mixture (1ml) was pipetted onto the column and eluted with hexane (25ml). Three eluates were collected with volumes of 1-5ml, 5-13ml and 13-25ml respectively. The eluants were concentrated, redissolved in iso-octane and examined using GC.

2.7. STABILITY OF 2,4-DICHLOROBENZYL ALKYL ETHERS TO LIGHT, TEMPERATURE AND AIR

2.7.1 Long Term Stability of a Solution of 2,4-Dichlorobenzyl Pentyl Ether

A solution of the pentyl ether ($1gl^{-1}$) in iso-octane was placed in screw-top vials (1ml). The test conditions are summarised in Table 2.5.

2.7.2 Long Term Stability of Pure 2,4-Dichlorobenzyl Alkyl Ethers to Different Conditions of Storage

The ethyl, hexyl, decyl and tetradecyl ethers (0.1g) were chosen as representative of the homologous series and sealed in glass ampoules. The conditions used are summarised in Table 2.6. The samples were examined after 9 months by GC-MS.

Table 2.5 Table showing conditions of storage for stability tests of 2,4-dichlorobenzyl pentyl ether solution.

Vial number	Stored over copper/no copper	Conditions of temperature and light	Method of vial cleaning
1	copper	laboratory light ambient temperature	hexane washed
2	copper	dark ambient temperature	hexane washed
3	copper	dark -5°C	hexane washed
4	no copper	laboratory light ambient temperature	hexane washed
5	no copper	dark ambient temperature	hexane washed
6	no copper	dark -5°C	hexane washed
7	copper	laboratory light ambient temperature	detergent washed
8	copper	dark ambient temperature	detergent washed
9	copper	dark -5°C	detergent washed
10	no copper	laboratory light ambient temperature	detergent washed
11	no copper	dark ambient temperature	detergent washed
12	no copper	dark -5°C	detergent washed

Table 2.6 Conditions of storage for 2,4-dichlorobenzyl alkyl ethers.

	Sample identity	conditions of storage	
		ampoule atmosphere	temperature
1	D2	argon	ambient temperature
2	D6	argon	ambient temperature
3	D10	argon	ambient temperature
4	D14	argon	ambient temperature
5	D2	air	ambient temperature
6	D6	air	ambient temperature
7	D10	air	ambient temperature
8	D14	air	ambient temperature
9	D2	argon	$-5^{o}C$
10	D6	argon	$-5^{o}C$
11	D10	argon	$-5^{o}C$
12	D14	argon	$-5^{o}C$
13	D2	air	$-5^{o}C$
14	D6	air	$-5^{o}C$
15	D10	air	$-5^{o}C$
16	D14	air	$-5^{o}C$

2.8 GAS CHROMATOGRAPHIC ANALYSIS OF 2,4-DICHLOROBENZYL ALKYL ETHERS

2.8.1 Preparation of Stock Solutions (Ether preparation described in 2.4.1)

2.8.1.1 Butyl, Hexyl, Octyl, Decyl, Dodecyl and Tetradecyl Ethers

Solutions of the above ethers (200mg l^{-1}) in iso-octane were prepared.

A composite mixture of the DCBEs (20mg l^{-1}) in iso-octane was prepared by serial dilution from the stock solutions for use with the GC-MS and a solution (0.1mg l^{-1}) was prepared for use with the GC-ECD. The three internal standards initially used for the work were tetrachloronaphthalene (TCN), octachloronaphthalene (OCN) and decachlorobiphenyl (DCBP).

2.8.1.2 Ethyl, Butyl, Hexyl, Heptyl, Octyl, Nonyl, Decyl, Undecyl, Dodecyl and Hexadecyl Ethers.

Solutions of each compound at 100mg l^{-1} in iso-octane were prepared. A composite mixture of the DCBEs (100mg l^{-1}) with tetrachloronaphthalene (0.05mg l^{-1}) as the internal standard was prepared for the GC-MS and at 0.2mg l^{-1} for the GC-ECD again with TCN as the internal standard (0.05mg l^{-1}).

2.8.2 Preliminary Calculation of Retention Indices

2.8.2.1 Solutions described in Section 2.8.1.1

Chromatograms of the standard DCBE solutions were obtained using the GC-ECD. Details of the chromatographic conditions are given in Table 2.7. The retention indices were calculated for the DCBEs using a series of n-alkyl trichloroacetates (ATA's) as retention index markers.[150]

Table 2.7 Gas chromatographic conditions for preliminary calculation retention indices.

Gas Chromatographic Parameters

Injector	on-column
Injector Temperature	ambient
Detector	Ni-63 electron capture
Detector Temperature	375°C
Carrier Gas	hydrogen
Carrier Gas Velocity	30-40cm sec^{-1}
Starting Temperature	120°C
Final Temperature	270°C
Temperature Programme	1 min isothermal 3 deg min^{-1} to 270°C 5 min isothermal
Column	CPSil5-CB

The alkyl tri-chloroacetate solution was chromatographed under similar conditions to the DCBEs and the assigned retention indices were used for each peak. A calibration curve of retention times versus retention indices was created for the ATAs using a fifth order polynomial least squares fit and the retention index values for DCBEs were calculated using this relationship. The polynomial relationship was calculated using the 'Curfit' programme.[154] The ATA solution and retention indices were supplied by Professor K. Ballschmiter.

2.8.2.2 Solutions described in Section 2.8.1.2

Chromatograms of the standard solutions prepared were recorded on the GC-MS. The chromatographic conditions used are summarised in Table 2.8. A standard solution of n-alkanes (C_{11}-C_{44} 50mg l^{-1}) was chromatographed under the same conditions.

Table 2.8 Gas chromatographic conditions for calculation DCBE retention indices on GC-MS.

Gas Chromatographic Parameters	
Injector	on-column
Injector Temperature	ambient
Detector	mass spectrometer
Carrier Gas	helium
Carrier Gas Flow Rate	55ml min^{-1}
Starting Temperature	110°C
Final Temperature	270°C
Temperature Programme	1 min isothermal 4 deg min^{-1} until all compounds eluted
Column	CPSil5-CB

The retention indices of the DCBE solution were calculated using the n-alkanes as calibration standards. A polynomial least squares fit was calculated as described in section 2.8.2.1 and the DCBE retention index values calculated as before. The standard alkane retention index values were assigned by nomenclature, ie ethyl = 200 RIU.

DCBE retention indices were also calculated using n-alkyl trichloroacetates as retention index standards as described in section 2.8.2.1.

2.8.2.3 Calculation DCBE Retention Indices at Different Temperature Programme Rates

Using the standard described in 2.8.1.1, chromatograms were recorded using the GC-MS. The conditions are summarised in Tables 2.9 and 2.10. Retention indices were calculated using the fifth order polynomial and a cubic spline fit developed by H Buchert and D E Wells.

Table 2.9 Gas chromatographic parameters used for GC-MS.

Gas Chromatographic Parameters

Injector	on-column
Detector	mass spectrometer
Carrier Gas	helium
Carrier Gas Flow Rate	55ml min^{-1}
Starting Temperature	110°C
Column	CPSil5-CB
Temperature Programme	see Table 2.10.

Table 2.10 Programme rates for comparison indices.

Sample identity	Concentration mg l^{-1}	Programme Rate Deg min-1
n-Alkanes	50	4
n-Alkanes	50	6
n-Alkanes	50	8
DCBEs	20	4
DCBEs	20	6
DCBEs	20	8

2.9. CALCULATION OF RETENTION INDICES FOR COMPLETE DCBE SERIES

2.9.1 Preparation of Standard Solutions and Calculation of Retention Indices for the DCBEs at Different Starting Temperatures and Programme Rates

A standard solution of each DCBE in iso-octane (200mg ml^{-1}) was prepared. Composite mixes for the GC-MS (10mg l^{-1}) and GC-ECD (0.5mg l^{-1}) were prepared by serial dilution. A mixture of the DCBEs and n-alkanes (50mg l^{-1}) in iso-octane was prepared for the GC-FID.

Using the alkane/DCBE mixture chromatograms were recorded using different starting temperatures and temperature programmes. Details of the injection and gas chromatographic conditions are given in Tables 2.11 and 2.12. In all cases an injection of 1μl was used and each injection was repeated at least three times. The conditions are shown for a CPSil5-CB column and the same conditions were used with a CPSil8-CB column.

Table 2.11 Details of gas chromatographic parameters for calculating retention indices.

Gas Chromatographic Parameters	
Injector	splitless
Injector temperature	275°C
Detector	flame ionization
Carrier Gas	hydrogen
Carrier Gas Velocity	35cm sec^{-1}
Starting Temperature	
Temperature Programme	see Table 2.12
Final Temperature	
Column	

Table 2.12 Details of the temperature programme rates, starting temperatures and columns used in the measurement of retention times for an alkane/DCBE mixture.[a,b,c]

Starting Temperature ^{o}C	Programme Rate $^{o}C\ min^{-1}$
80	3
Ditto	5
Ditto	7
100	3
Ditto	5
Ditto	7
120	3
Ditto	5
Ditto	7

a. Temperature gradient, starting temperature held for 1 minute, then temperature programme to 260^{o}C, then temperature programme of 30 deg min^{-1} up to a final temperature of 290^{o}C.

b. Sample Alkane DCBE mixture

c. Column CPS115-CB

CPS118-CB

The alkanes and DCBEs were chromatographed in a composite mixture and the retention indices of the DCBEs calculated using the alkanes as standards and a cubic spline programme.

2.9.2 Calculation of Retention Indices of Organochlorine Compounds at Different Starting Temperatures and Temperature Programme Rates

Retention indices were calculated for solutions of organochlorine compounds. The content of each solution is described in Table 2.13. The structure of each compound is shown in the appendix 1.

Retention indices were calculated for the S1 and S2 solutions using the DCBEs as retention index standards at different temperature programme rates and starting temperatures. The conditions are summarised in Table 2.14. Retention indices were calculated by recording chromatograms of the DCBE standard solutions and organochlorine solutions under identical conditions.

Chromatograms were recorded for the S1 and S2 organochlorine standard solutions using starting temperatures of 80°C, 100°C or 120°C and temperature programme rates of 3 deg min^{-1} or 5 deg min^{-1} on two columns, a CPSil5-CB or a CPSil8-CB column.

A calibration curve was created for the DCBE peaks using RI values calculated in section 2.9.1 and a cubic spline least squares fit. Using the relationship, retention indices were calculated for the organochlorine solutions. Two DCBE internal standards were present in each organochlorine solution.

Table 2.13 Consitutent compounds in the organochorine standard solutions. (Structures are shown in appendix 1.)

S1	S2
Hexachlorobenzene (HCB)	α-Hexachlorocyclohexane (αHCH)
PCB 28	β-Hexachlorocyclohexane (βHCH)
Heptachlor	γ-Hexachlorocyclohexane (γHCH)
D7	Heptachlor
PCB 52	D7
Aldrin	α-Chlordene
PCB 44	γ-Chlordene
2,4 DDE	Heptachlor epoxide
PCB 101	Oxychlorodane
4,4 DDE	γ-Chlordane
PCB 118	Endosulphan 1
PCB 153	2,4 DDE
PCB 137	α-Chlordane
PCB 138	Trans nonachlor
PCB 128	Dieldrin
PCB 180	2,4 DDD
Mirex	Endrin
D14	4,4 DDD
PCB 195	2,4 DDT
PCB 194	4,4 DDT
	D12
	PADS
	cis permethrin
	trans permethrin

PCB numbering after Ballschmiter[150].

Table 2.14 Gas chromatographic conditions for calculation of organochlorine retention indices.

Gas Chromatographic Parameters

Injector		on column
Injector Temperature		ambient
Detector		electron capture
Detector Temperature		375°C
Carrier Gas		Hydrogen
Carrier Gas Velocity		35cm sec^{-1}
Starting Temperature		120°C
Final Temperature		260°C
Temperature Programmes	a	1 minute isothermal, 3 deg min^{-1} to 260°C, 5 minutes isothermal
	b	1 minute isothermal, 5 deg min^{-1} to 260°C, 5 minutes isothermal
Column		CPSil5-CB and CPSil8-CB

2.9.3 Calculation of Retention Indices for Unknown Samples and Quantitation of Peaks

A DCBE calibration mixture was chromatographed followed by an organochlorine standard and the unknown sample under the same chromatographic conditions. Retention indices were assigned to each DCBE peak and using the cubic spline routine, retention indices were calculated for the organochlorine standards and unknowns. Comparison of the two sets of retention index values allowed qualitative peak identification. A retention index 'window' of three units was used.

Quantitation was obtained by the use of two bracketing organochlorine standard solutions containing approximately 0.02mg l^{-1} and 0.1mg l^{-1} of each compound and the programme developed by Buchert and Wells. The DCBE internal standards were present at 0.5 mg l^{-1}.

The content of each standard solution is shown in Table 2.13.

2.10 GEL PERMEATION CHROMATOGRAPHY

The gel permeation equipment was assembled as shown in Figure 2.1.

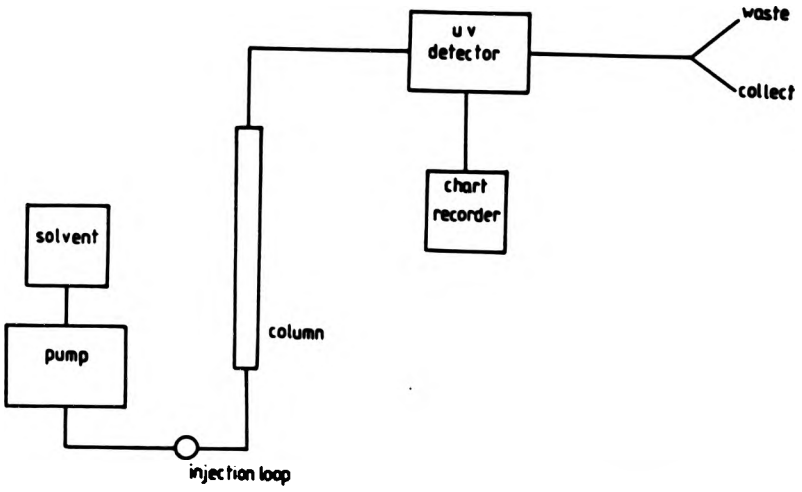

FIGURE 2.1 Schematic diagram of gel permeation equipment.

2.10.1 Preparation of Gel Permeation Chromatography (GPC) Columns

The gel permeation chromatographic column was prepared as reported by Hopper et al.[153] SX-3 biobeads (40-50g) were soaked overnight in a solution of dichloromethane/hexane 50:50 (200ml). The resulting slurry was poured into a column (25mm i.d. x 30cm length) and allowed to settle for twenty minutes. Excess solvent was removed from the head of the column and the column length adjusted to 18cm. The packing was again allowed to settle and excess solvent removed. The column end fittings were adjusted without compressing the column packing to minimise the dead volume. The column was conditioned by pumping at $1ml/min^{-1}$ dichloromethane/hexane through it for five hours. The column was conditioned prior to each use by eluting with solvent for one hour.

2.10.2 Preparation of Test Samples of Fish Oils and Organochlorine Compounds

A test solution containing highly refined cod liver oil ($100mg/ml^{-1}$), hexachlorobenzene ($20mgml^{-1}$) and decachlorobiphenyl ($20mgml^{-1}$) in hexane/dichloromethane (50/50 v.v) was prepared. 1ml was injected at flow rates of $1ml\ min^{-1}$, $2ml\ min^{-1}$, $3ml\ min^{-1}$, $4ml\ min^{-1}$ and $5ml\ min^{-1}$. The lipid profile was examined by uv spectrometry at 254nm and 1 AUF.

2.10.3 Identification and Measurement of Lipid Residues in Fish Oils

A stock solution of highly refined cod liver oil ($200mg/ml^{-1}$) in hexane was prepared and diluted to $100mgml^{-1}$ with dichloromethane before use.

The non-volatile lipid residue in the highly refined cod liver oil was measured by injecting 1ml and collecting two fractions between 0-33ml

and 34-134ml. The eluates were collected in pre-weighed evaporating dishes, the solvent evaporated and the dishes weighed to constant weight at room temperature. The procedure was repeated three times.

A fresh solution of highly refined cod liver oil was prepared and the above procedure repeated. The residues present in the fraction 36-90ml were measured along with the residues in the fraction 0-36ml and 36-55ml.

2.10.4 Measurement of Organochlorine Residues Present in Fish Oils

Highly refined cod liver oil (100mg ml^{-1}, 1ml) was injected onto the GPC column and the separation monitored using the uv detector. After the lipid fraction had eluted (83ml eluate), a second fraction (50ml) was collected and the samples prepared for gas chromatographic analysis (see section 2.10.14). Four replicate injections were made; 2 were used for determination of lipid content and 2 to determine the organochlorine content. System blanks were also examined for organochlorine residues as follows:

(a) Solvent (50ml) from the gel permeation system was collected and concentrated for GC analysis without further treatment.

(b) Solvent (50ml) from the gel permeation was collected and subjected to the preparation described in section 2.10.12.

(c) Solvent (50ml) was concentrated to 1ml and analysed by GC.

2.10.5 Preparation of Test and Stock Solutions of Refined Capelin Oil

A test solution containing refined capelin oil (100mg ml^{-1}), hexachlorobenzene (20mg l^{-1}) and decachlorobiphenyl (20mg l^{-1}) in hexane/dichloromethane (50/50 v.v) was prepared. A stock solution containing refined capelin oil (200mg l^{-1}) in hexane was prepared and diluted with dichloromethane (50/50 v/v) before use.

2.10.6 Test Injections and Measurement of Lipid Residues in Refined Capelin Oil

Details of the test injections are given in Table 2.15 using an injection volume of 1ml. The lipid residues were measured as described in section 2.10.3. The residues in each of the two lipid peaks and the lipid residues which co-elute with the early eluting organochlorines were measured.

Two fractions were collected, those from 37-70ml and 70-170ml. The above method was repeated and the fractions 37-84ml and 84-184ml collected.

Table 2.15 Details of test injections of fish oils described in 2.10.6.

Sample Identity	Sample Concentration mg l^{-1}	Flow Rate (ml min^{-1})	Fraction Collected (ml)
Refined Capelin Oil	100	3	none
Refined Capelin Oil + HCB + DCBP	100, 20, 20	1	Ditto
Ditto	Ditto	2	Ditto
Ditto	Ditto	3	Ditto

2.10.7 Preparation of Test and Stock Solutions of Fish Oils

Solutions of highly refined cod liver oil (400mg ml^{-1}) and refined capelin oil (400mg ml^{-1}) in hexane (50ml) were prepared and diluted to 200mg ml^{-1} with dichloromethane before use.

A test solution of highly refined cod liver oil (200mg ml^{-1}), hexachlorobenzene (20mg l^{-1}) and decachlorobiphenyl (20mg l^{-1}) in

dichloromethane/hexane was prepared.

2.10.8 Test Injections and Measurement of Lipid Residues in Refined Capelin Oil and Highly Refined Cod Liver Oil

The test solution described in 2.10.7 containing 200 mg ml^{-1} refined capelin oil was injected (1ml) using a flow rate of 3ml min^{-1}. Two fractions were collected between 36-108ml and 108-208ml.

A test injection (1ml) of the highly refined cod liver oil was made and no eluant collected. An injection of the solution containing highly refined cod liver oil, hexachlorobenzene and decachlorobiphenyl was also made.

2.10.9 Preparation of Fish Oil Solutions

Highly refined cod liver oil (HRCLO), refined capelin oil (RCO), crude cod liver oil (CCLO) and crude capelin oil (CCO) each at 400mg l^{-1} in hexane (25ml) were prepared.

2.10.10 Test Injections and Measurement of Lipid Residues in Fish Oils

Using the solutions described in 2.10.9 and 20 mgl^{-1} solutions of hexachlorobenzene and decachlorobiphenyl, test injections were made to identify the elution characteristics of the organochlorine components. The injection volume used was 2ml and the flow rate 3ml min^{-1}. The non-volatile lipid residues were measured by collecting the fraction eluting between 90 and 140ml for each of the four fish oils. This procedure was repeated in triplicate, and the fish oils were evaporated to dryness and weighed as described in section 2.10.3.

2.10.11 Measurement of Organochlorine Residues in Fish Oils

Triplicate injections of the four fish oils were made using an injection volume of 2ml and a flow rate of 3ml min^{-1}. The concentration of each of the fish oils was 200mg ml^{-1}.

The fraction eluting between 90 and 190ml was collected for subsequent analysis.

2.10.12 Preparation of Samples for Examination by Gas Chromatography

Eluates (100ml) collected from the gel permeation column (as described in 2.10.8) were concentrated to 1ml prior to separation on alumina and silica columns. Duplicate blank samples of solvent were also prepared using these columns.

The alumina columns were prepared as described in section 2.6.4. The samples were eluted with hexane (60ml) and the following eluates collected:

Eluate 1 1-6ml

Eluate 2 6-54ml

Eluate 1 was concentrated to 1ml and subjected to further separation on a silica column. Each column contained silica (3g) and the sample was pipetted onto the column as before. The sample was eluted with hexane (15ml) and the eluates collected were:

Eluate 1A 1-6ml

Eluate 1B 6-15ml

Eluate 1B was combined with eluate 2 from the alumina column, and evaporated to 0.2ml, diluted with iso-octane to 5ml and reconcentrated. This was repeated twice to remove as much hexane as possible. The final volume of the sample was adjusted to 1ml. Eluate 1A from the silica column was reconcentrated and re-dissolved in iso-octane in a similar method.

An internal standard solution containing the heptyl and tetradecyl ethers at 0.5mg l^{-1} using the heptyl (D7) and tetradecyl (D14) ethers was added to eluate 1A and D_7, D_{12} to eluate 1B+2. The samples were reconcentrated to 1ml and analysed by open tubular gas chromatography.

2.10.13 Examination of Fish Oil Samples using Open Tubular Gas Chromatography

The gas chromatographic conditions used are summarised in Table 2.16. The data were collected and analysed using an Apple IIe 64K micro-computer interfaced to the gas chromatograph using an Adalab A/D interface card and the 'Chromatochart' program.

The samples were chromatographed on two columns, a CPSil5-CB and a CPSil8-CB column using the same conditions.

Table 2.16 Details of gas chromatographic conditions used for the analysis of fish oils.

G C Parameters

Injector	splitless
Injector Temperature	275°C
Detector	Ni-63 electron capture
Detector Temperature	350°C
Carrier Gas	Hydrogen
Carrier Gas Velocity	30-40cm sec^{-1}
Starting Temperature	80°C
Final Temperature	270°C
Temperature Programme	1 min isothermal, 3 deg min^{-1} to 260°C, 5 min isothermal

2.10.14 Calculation of Retention Indices, Assignment of Peak Identities and Quantitation of Peaks

The calculation of retention indices, assignment of peak identities and quantitation of peaks is described in section 2.9.3.

CHAPTER THREE

RESULTS

3.1 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) OF 2,4-DICHLOROBENZYL ALKYL ETHERS

3.1.1 Decyl, Dodecyl, Tetradecyl and Octadecyl Ethers

The preparation of these compounds is described in sections 2.3.1 (octadecyl ether) and 2.3.2 (tetradecyl, dodecyl and decyl ethers) of Chapter 2.

The gas chromatograms of the above compounds are shown overleaf in Figures 3.1, 3.2, 3.3 and 3.4. The chromatograms were recorded using a temperature programme of 10 deg min^{-1} on a CPSil5-CB column.

3.1.2 GC-MS of Ether Solution Described in Section 2.8.1.1, Chapter 2

The ethers were prepared as described in section 2.4.1 and a gas chromatogram of the composite mix is as shown in Figure 3.5.

3.1.3 GC-MS of Ether Solution Described in Section 2.8.1.2

The gas chromatogram of the composite mixture containing the ethyl, butyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and hexadecyl ethers is shown in Figure 3.6.

3.1.4 GC-MS of Complete DCBE Mixture

A chromatogram of the solution containing the ethyl-hexadecyl ethers described in section 2.9.1 is shown in Figure 3.7 and the mass spectral data are shown in Table 3.1. A representative mass spectrum of the tetradecyl ether is shown in Figure 3.8.

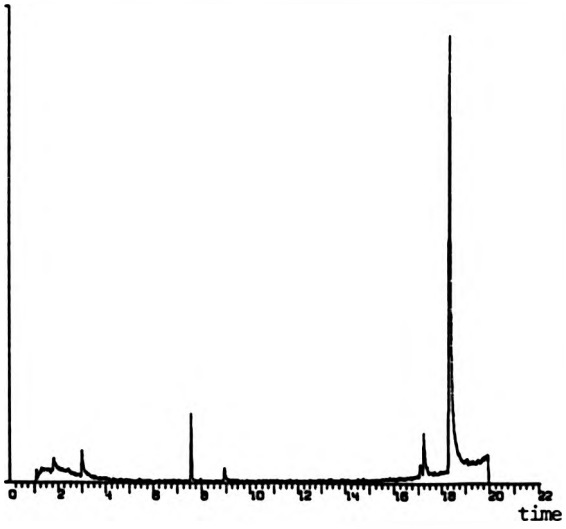

FIGURE 3.1 Chromatogram of 2,4-dichlorobenzyl octadecyl ether.

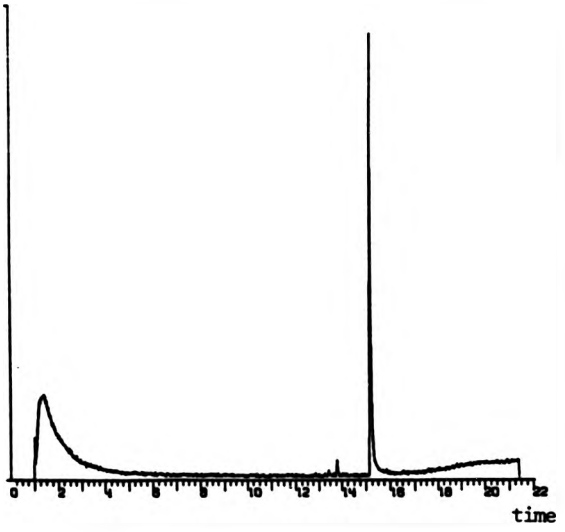

FIGURE 3.2 Chromatogram of 2,4-dichlorobenzyl tetradecyl ether.

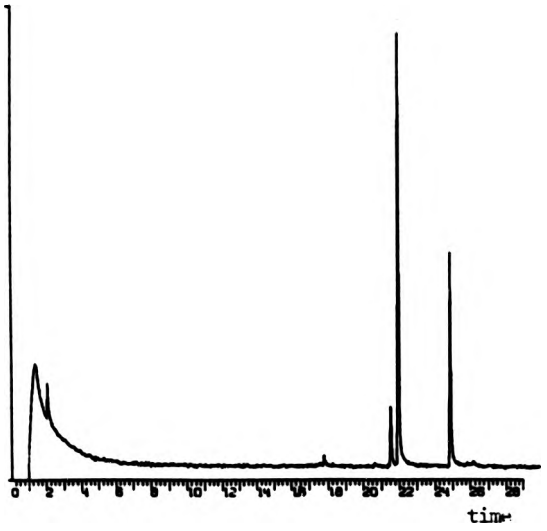

FIGURE 3.3 Chromatogram of 2,4-dichlorobenzyl dodecyl ether.

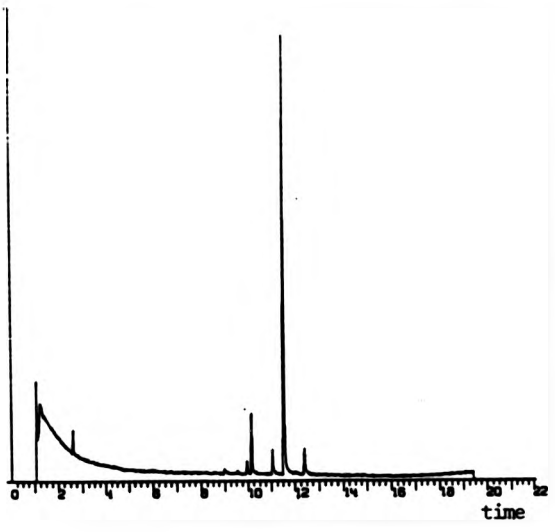

FIGURE 3.4 Chromatogram of 2,4-dichlorobenzyl decyl ether.

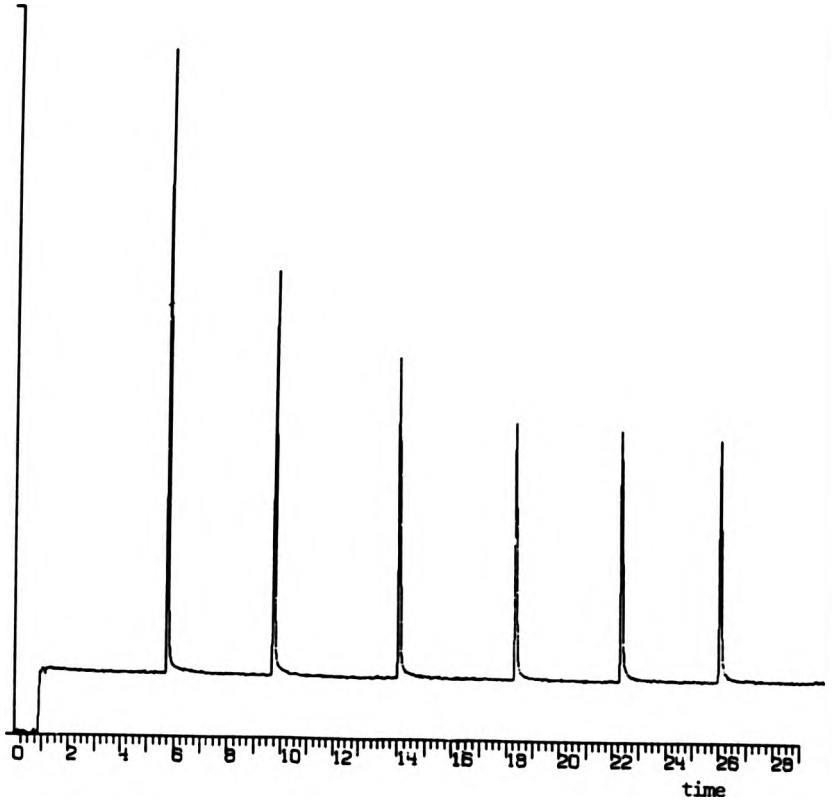

FIGURE 3.5 Chromatogram of solution described in 3.1.2.

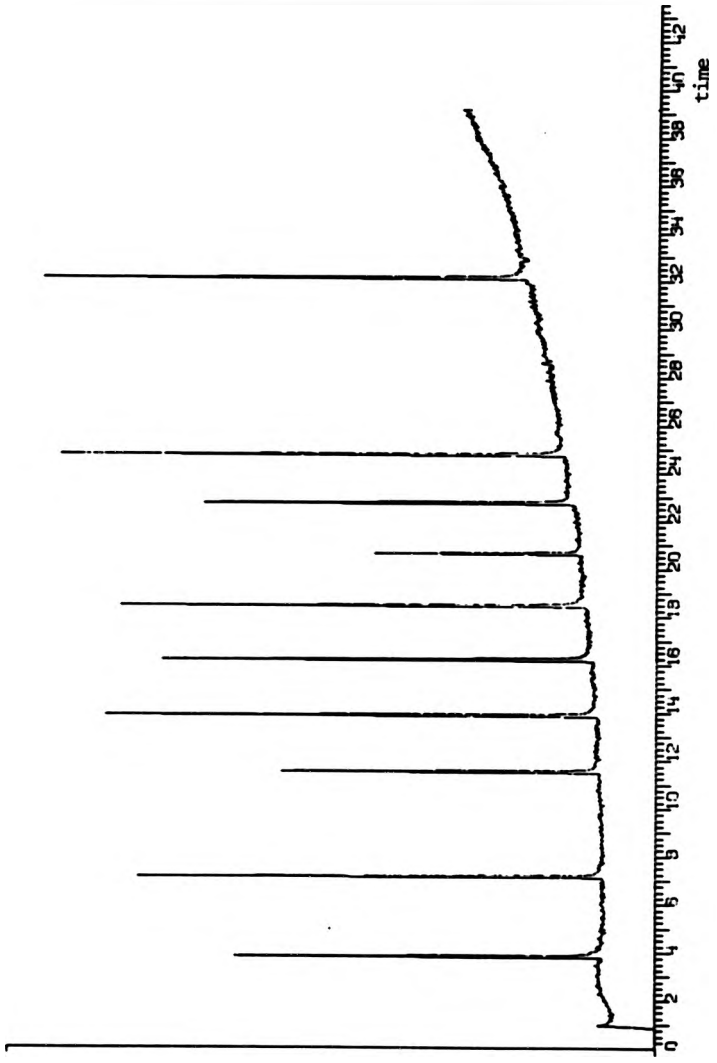

FIGURE 3.6 Chromatogram of composite mixture described in Section 3.1.3.

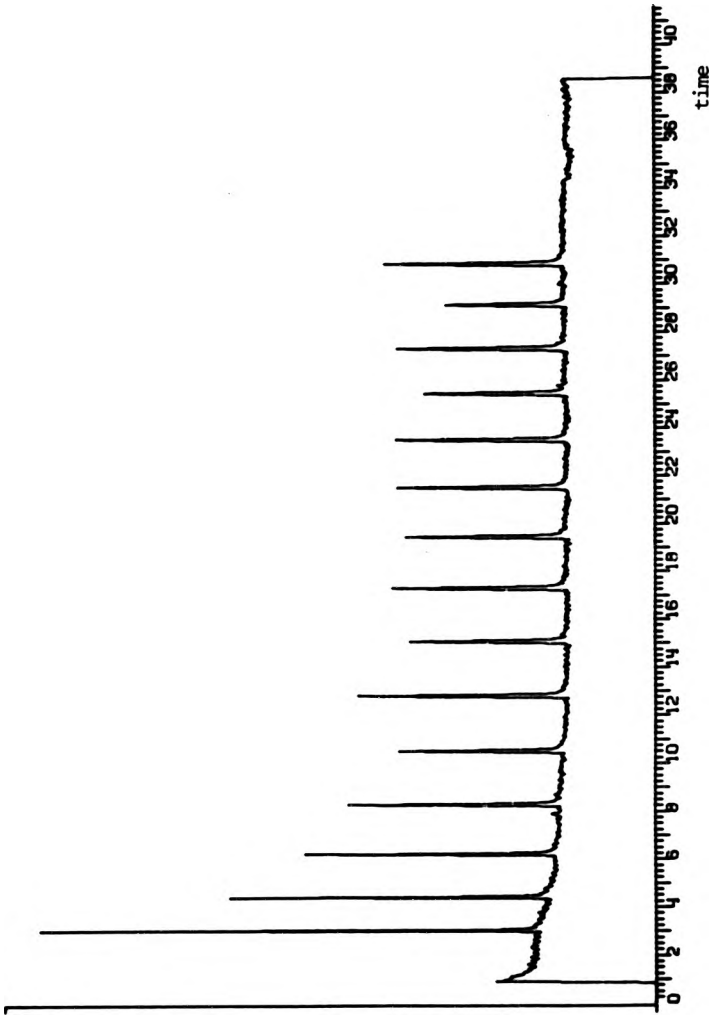

FIGURE 3.7 Chromatogram of composite mixture described in Section 3.1.4.

Table 3.1 Mass Spectral Data for 2,4-Dichlorobenzyl Alkyl Ethers.

Compound	Ion m/z / Abundance %												
Ethyl	204*	169	159	141	125	123	113	111	89	75	57		
	18	35	100	38	32	20	20	31	15	15	18		
Propyl	218*	183	159	141	125	123	111	89	75	63			
	8	15	100	22	13	13	8	15	8	8			
Butyl	232*	197	159	141	125	123	111	89	75	63	57		
	1	9	100	20	15	13	8	15	8	8	10		
Pentyl	246*	159	141	125	123	111	89	87	75	69	63		
	1	100	15	15	12	5	12	15	7	50	8		
Hexyl	260*	159	141	125	123	101	89	83	82	63	55		
	1	100	10	12	11	12	15	52	7	8	40		
Heptyl	274*	180	176	159	141	125	123	115	97	89	69	63	57	55
	1	8	7	100	15	15	13	15	59	17	15	7	8	78
Octyl	288*	178	159	141	129	125	123	111	89	69	57	55		
	1	8	98	9	10	12	11	21	14	100	32	22		
Nonyl	302*	176	159	143	141	125	123	89	83	69	57	55		
	1	10	100	12	10	18	11	15	55	98	23	35		
Decyl	316*	176	159	141	125	123	97	89	83	71	69	57	55	
		10	100	10	16	10	40	11	90	15	40	40	38	
Undecyl		176	159	125	111	97	89	83	71	69	57	55		
		10	100	15	21	71	15	60	21	45	38	40		
Dodecyl		159	125	123	111	97	89	85	83	71	69	57	55	
		100	20	10	38	60	12	17	52	20	58	45	38	
Tridecyl		176	159	125	111	97	83	71	69	57	55			
		11	100	28	33	52	58	18	60	45	41			
Tetradecyl		176	159	125	111	97	85	83	71	69	57	55		
		12	100	30	33	60	13	65	28	58	58	48		
Pentadecyl	386*	176	159	141	125	111	97	85	83	71	69	57	55	
		11	100	11	30	33	65	15	65	25	37	57	40	
Hexadecyl	400*	176	159	125	111	97	85	83	71	69	57	55		
		13	100	33	42	72	18	65	40	42	67	42		

All spectra recorded at 70 eV.

* Molecular Ion

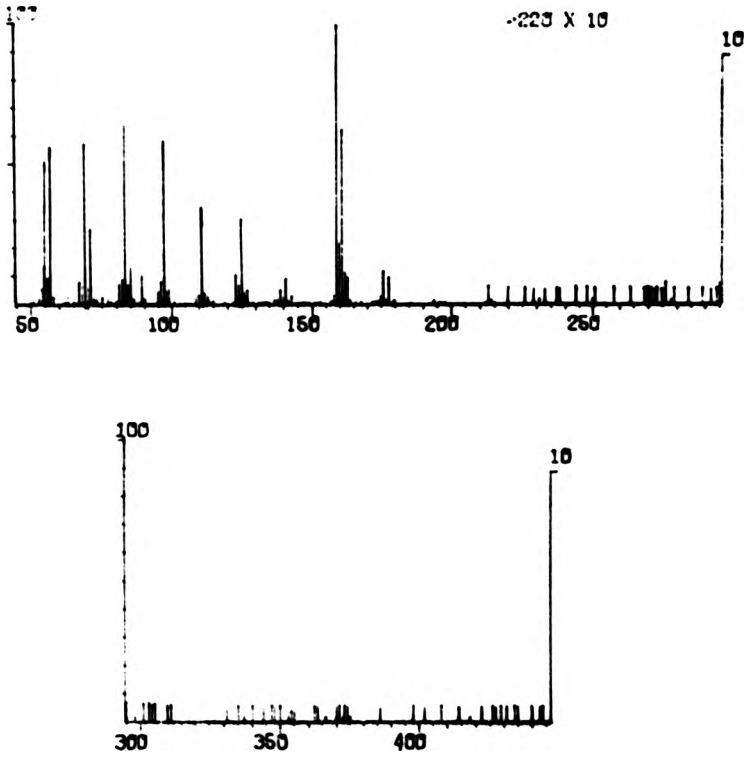

FIGURE 3.8 Mass spectrum of 2,4-Dichlorobenzyl Tetradecyl Ether.

3.2 STABILITY OF 2,4-DICHLOROBENZYL ALKYL ETHERS

3.2.1 Stability of DCBEs to Laboratory Methods used for the Chemical Clean-up of Liquid Extracts (described in Section 2.6)

3.2.1.1 Concentrated H_2SO_4

On examination using gas chromatography, the DCBE's had degraded chemically. No attempt was made to identify the breakdown products.

3.2.1.2 Alcoholic KOH

The DCBEs were unaffected by the reaction.

3.2.1.3 Methyl Iodide

The DCBEs were unaffected by reaction with methyl iodide.

3.2.1.4 Alumina Column

The DCBEs eluted in eluate 2 and were not chemically affected by the column.

3.2.2 Stability of DCBEs to Light Temperature and Air

3.2.2.1 Long Term Stability of Solution of 2,4-Dichlorobenzyl Pentyl Ether (described in Section 2.7.1)

The $1000mgl^{-1}$ solution of the pentyl ether showed no degradation under the conditions listed in Table 2.5 over a period of 9 months.

3.2.2.2 Long Term Stability of 2,4-Dichlorobenzyl Alkyl Ethers (described in Section 2.7.2)

The compounds stored under argon both at ambient temperatures and at $-5^{o}C$ showed no degradation under examination by GC-MS a solution of concentration of $200mgl^{-1}$ was used. The compounds stored under air at $-5^{o}C$ also showed no degradation.

The chromatograms for the ethyl, hexyl, decyl and tetradecyl ethers stored under air at ambient temperatures are shown in figures 3.9, 3.10, 3.11 and 3.12.

The ethyl ether appeared to show no degradation and the tetradecyl ether showed no trace of the acid or aldehyde.

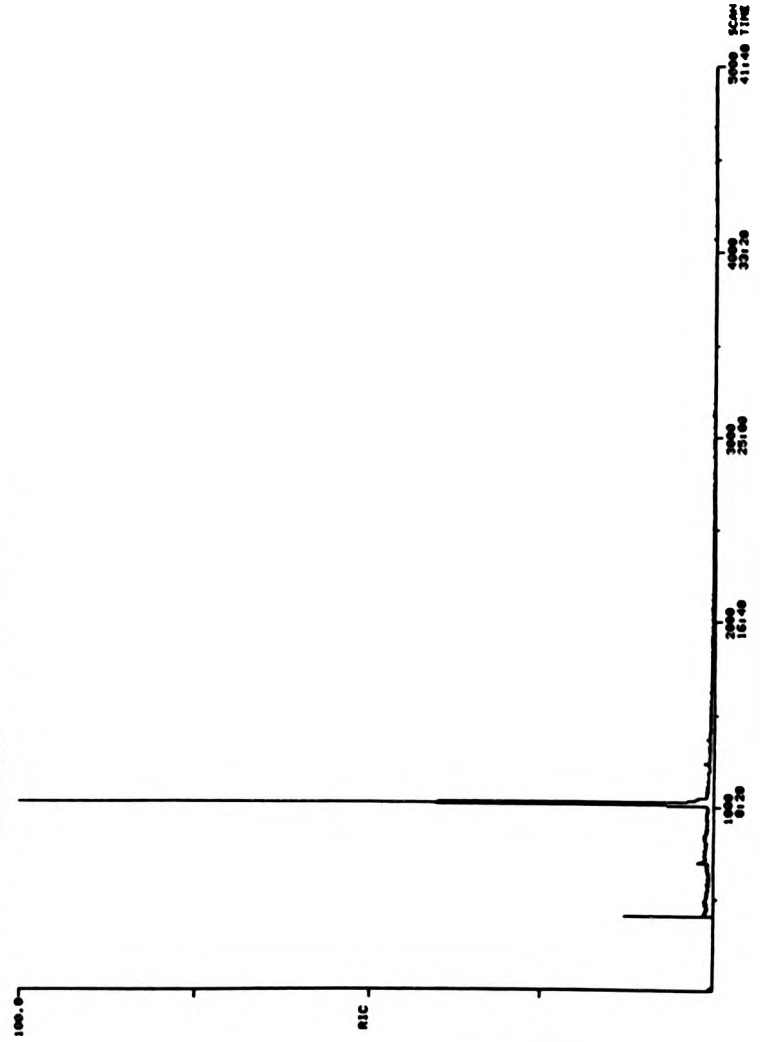

FIGURE 3.9 Chromatogram of 2,4-dichlorobenzyl ethyl ether after storage under air at ambient temperature.

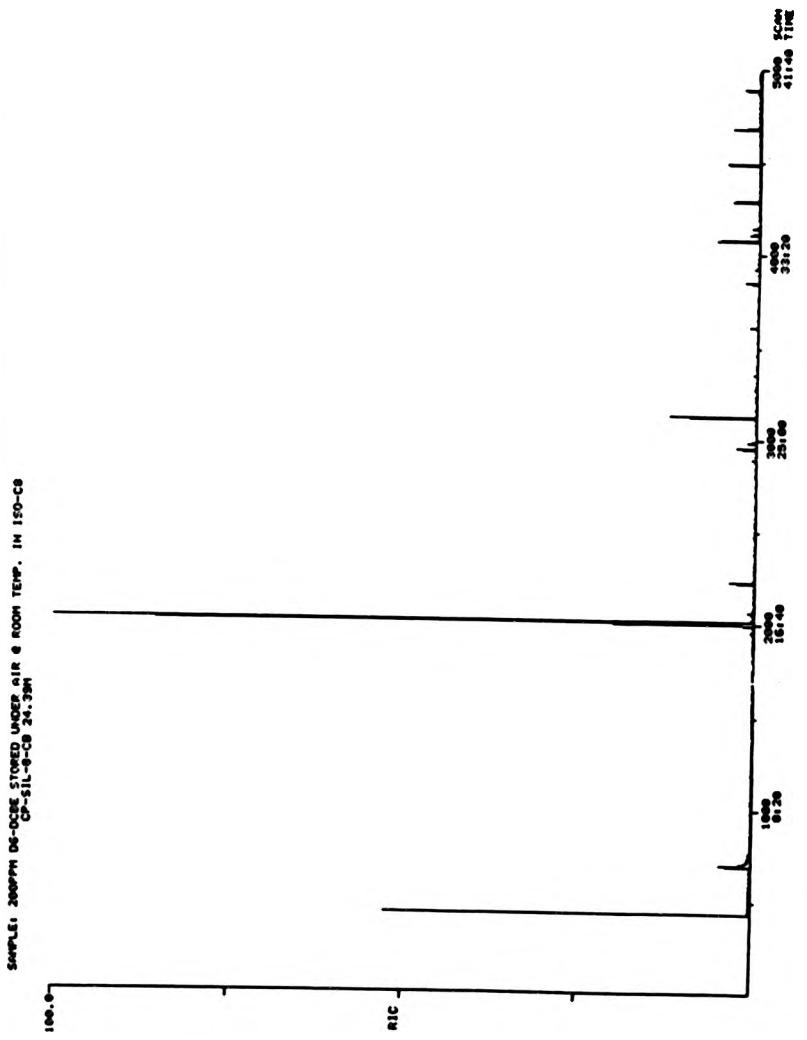

FIGURE 3.10 Chromatogram of 2,4-dichlorobenzyl hexyl ether after storage under air at ambient temperature.

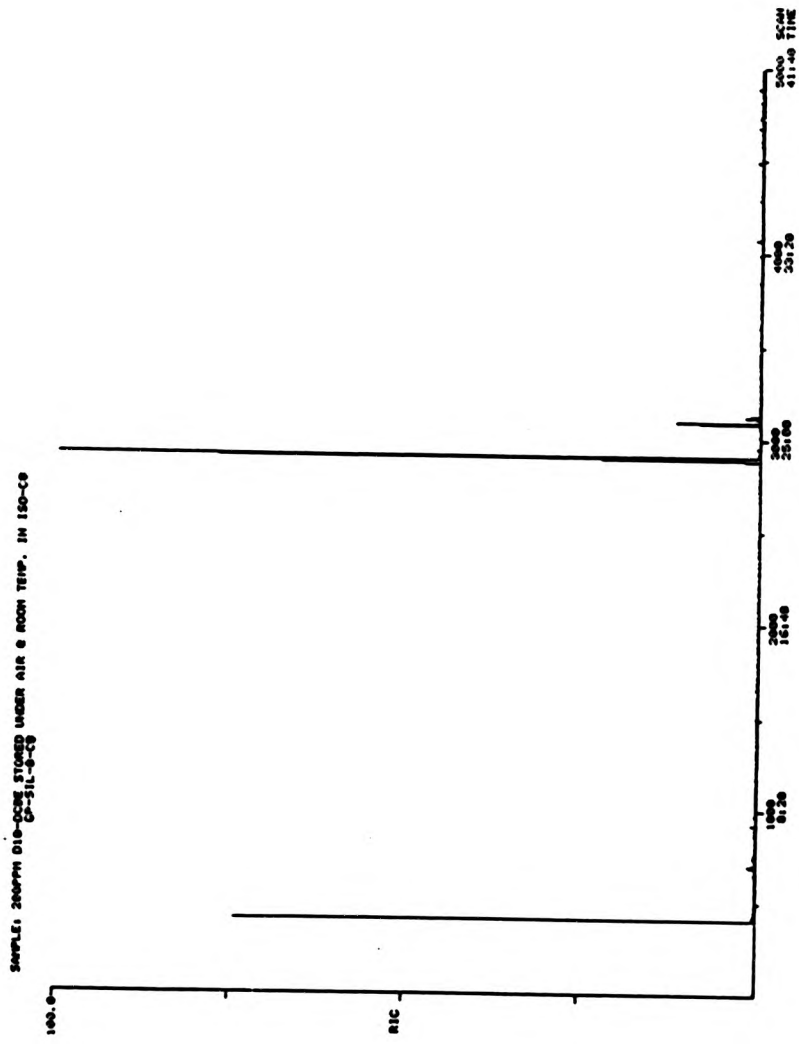

FIGURE 3.11 Chromatogram of 2,4-dichlorobenzyl decyl ether after storage under air at ambient temperature.

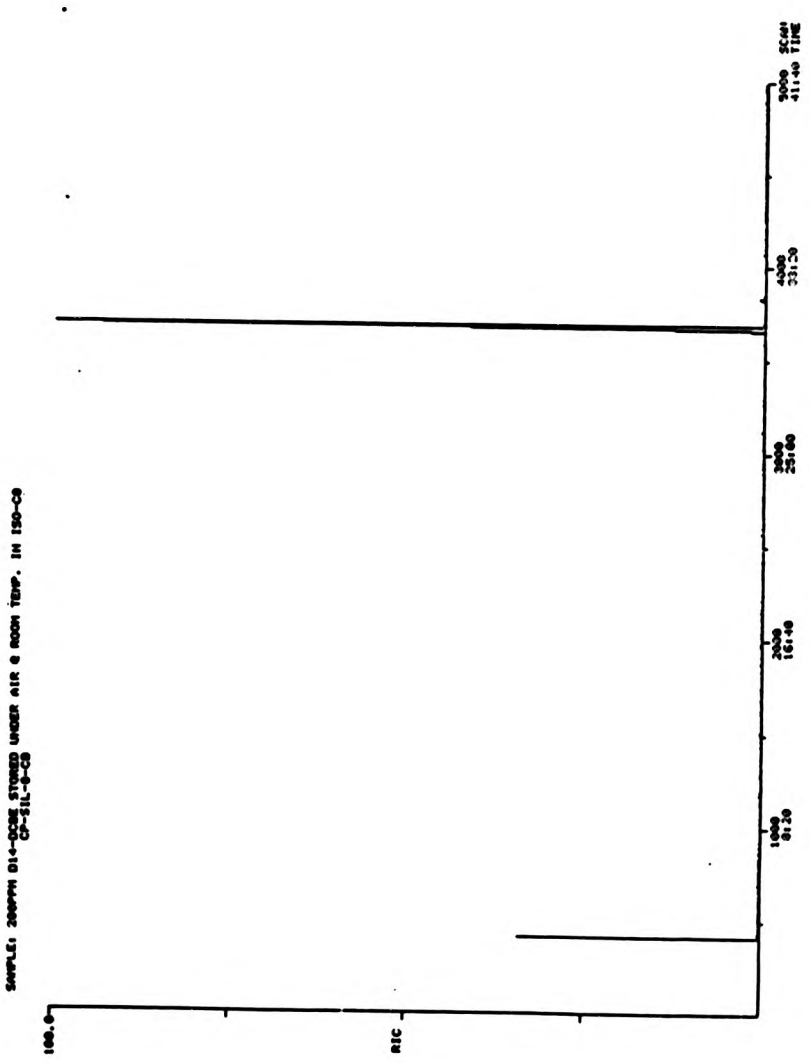

FIGURE 3.12 Chromatogram of 2,4-dichlorobenzyl tetradecyl ether after storage under air at ambient temperature.

3.3 CALCULATION OF RETENTION INDICES

3.3.1 Alkyl Trichloroacetates (ATAs) as Retention Index Standards

Chromatograms were recorded for the solution containing the butyl, hexyl, octyl, decyl, dodecyl and tetradecyl ethers (described in Section 2.8.1.1) using the GC-ECD. The retention times are summarised in Table 3.2. The retention indices were calculated by means of a fifth order polynomial function using the Curfit programme and the alkyl trichloroacetates (ATAs) as retention index standards (as described in Section 2.8.2.1). The ATA retention times are summarised in Table 3.3.

The values of the retention indices were provided by Professor K Ballschmiter (University of Ulm). The internal standards used were tetrachloronaphthalene (TCN), octachloronaphthalene (OCN) and decachlorobiphenyl (DCBP).

Table 3.2 Retention times and indices of DCBEs using ATAs as standards.

DCBE	Retention Time (sec)[a]		Retention Index	
D4	426.4	429.5	1613.1	1615.2
D6	740.1	744.7	1813.5	1816.2
D8	1100.2	1104.8	2016.3	2018.8
D10	1460.8	1465.8	2215.7	2218.5
D12	1804.5	1809.6	2415.4	2418.5
D14	2127.4	2131.9	2620.0	2623.0
TCN	999.1	1005.5	1960.5	1964.0
OCN	2338.4	2345.0	2763.5	2768.1
DCBP	2450.1	2455.6	2841.4	2845.3

a Values from two injections are presented.

Table 3.3 Retention times and indices of alkyl trichloroacetates.

Carbon number of alkyl residue of ATA	Retention index	Retention time (sec)
7	1500	288.4
8	1600	405.2
9	1700	550.4
10	1800	715.2
11	1900	893.6
12	2000	1074.2
13	2100	1256.5
14	2200	1434.0
15	2300	1607.2
16	2400	1775.7
17	2500	1937.2
18	2600	2095.4
19	2700	2247.5
20	2800	2395.2
21	2900	2537.2
22	3000	2676.5

3.3.2 Retention Indices of DCBEs Calculated Using Alkanes as Retention Index Standards

Retention indices were calculated for the DCBE solution described in Section 2.8.1.2, Chapter 2, containing the ethyl, butyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and hexadecyl ethers. The retention index standards were the n-alkanes and the chromatograms were recorded using the GC-MS. The retention times and indices of the n-alkanes are shown in Table 3.4 and those of the DCBEs in Table 3.5. A graph of the calculated retention index versus the carbon number of the DCBE alkyl chain is shown in Figure 3.13. A linear function gave correlation coefficient of 0.9999 and a standard error of 5.9597 index units.

Table 3.4 Retention times and indices of n-alkanes.

n-alkane	Retention index	Retention time (sec)
C11	1100	81
C12	1200	118
C13	1300	176
C14	1400	257
C16	1600	480
C18	1800	750
C20	2000	1020
C22	2200	1272
C24	2400	1503
C26	2600	1713
C28	2800	1913
C30	3000	2102
C36	3600	2613

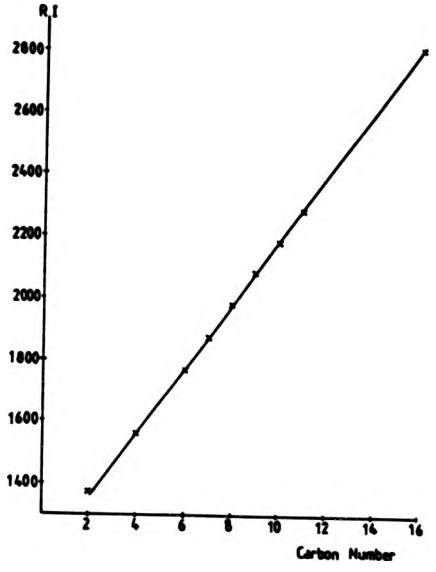

FIGURE 3.13 Graph of retention index versus carbon number of alkyl chain of DCBEs described in Section 3.3.2.

Table 3.5 Retention indices and retention times of DCBEs.

DCBE	Carbon number of alkyl chain	Retention time (sec)	Retention Index
D2	2	234	1371
D4	4	439	1562
D6	6	795	1769
D7	7	845	1874
D8	8	984	1979
D9	9	1119	2080
D10	10	1249	2181
D11	11	1371	2282
D12	12	1488	2383
D16	16	1922	2810

3.3.3 Retention Indices of DCBEs Using ATAs as Retention Index Standards at Different Programme Rates

For the DCBE solution containing the ethyl, butyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and hexadecyl ethers described in Section 2.8.1.2, Chapter 2, Retention indices were calculated using the ATAs as retention index markers. The retention times and indices of the ATAs are given in Table 3.6 and those for the DCBEs are given in Table 3.7.

A fifth order polynomial was fitted giving a correlation coefficient of 0.99999 and a standard error of 2.8674 index units.

3.3.4 Retention Indices of DCBEs Using n-Alkanes as Retention Index Standards at Different Temperature Programme Rates

Using the solutions described in Section 2.8.1.2, retention indices were calculated for the DCBEs at different temperature programme rates.

The retention times of the n-alkanes are given in Table 3.8 and those for the DCBEs in Table 3.9. The retention indices for the DCBEs were calculated using a polynomial and a cubic spline function. A comparison of the retention index values is given in Table 3.9.

Table 3.6 Retention times and retention indices of ATAs.

ATA	Retention index	Retention time (sec)
6	1400	147.6
7	1500	261.6
8	1600	391.4
9	1700	530.1
10	1800	689.8
11	1900	862.8
12	2000	1042.1
13	2100	1221.3
14	2200	1396.2
15	2300	1569.3
16	2400	1736.9
17	2500	1898.3
18	2600	2055.1
19	2700	2205.1
20	2800	2352.6
21	2900	2494.3
22	3000	2631.5

Table 3.7 Retention times and retention indices of DCBEs.

	Retention time (sec)	Retention Index
D2	214.4	1462.3
D4	407.3	1614.6
D6	709.1	1809.8
D7	883.4	1910.9
D8	1062.4	2012.6
D9	1263.4	2114.3
D10	1419.1	2213.7
D11	1591.2	2313.0
D12	1760.2	2414.6
D16	2382.7	2822.7

Table 3.8 Retention times and retention indices of n-alkanes at different temperature programmes.

n-Alkane	Retention Index	Retention Time (sec)		
		4 deg min^{-1}	6 deg min^{-1}	8 deg min^{-1}
C12	1200	79.2	79.2	*
C13	1300	127.8	124.2	118.8
C14	1400	201.6	187.2	174.6
C16	1600	410.4	356.4	313.2
C18	1800	673.2	549.0	464.4
C20	2000	943.2	741.6	608.4
C22	2200	1198.8	918.0	741.6
C24	2400	1434.6	1080.0	864.0
C26	2600	1652.4	1229.4	977.4
C28	2800	1862.4	1373.4	1083.6
C30	3000	2058.6	1506.6	1184.4
C32	3200	2244.0	1634.4	1278.0
C36	3600	2584.2	1869.6	

* elution too rapid to measure peak.

Table 3.9 Retention indices of DCBEs for different temperature programmes using polynomial and cubic spline functions.

DCBE	4 deg min^{-1}		6 deg min^{-1}		8 deg min^{-1}	
	Polynomial	Cubic Spline	Polynomial	Cubic Spline	Polynomial	Cubic Spline
D2	1364	1369	1363	1369	1364	1366
D4	1552	1558	1557	1561	1556	1562
D6	1760	1761	1765	1763	1766	1764
D7	1866	1862	1870	1866	1872	1868
D8	1970	1965	1973	1967	1975	1972
D9	2073	2067	2076	2071	2079	2076
D10	2176	2172	2179	2177	2181	2180
D11	2278	2276	2280	2280	2282	2283
D12	2380	2381	2381	2384	2385	2388
D14	2587	2593	2594	2600	2596	2600
D16	2798	2800	2800	2800	2801	2800

3.4 RETENTION INDICES OF COMPLETE DCBE SERIES

3.4.1 Retention Times and Retention Indices of DCBEs and n-Alkanes.

Retention indices were calculated for the solution of DCBEs containing the ethyl-hexadecyl ethers described in Section 2.9.1. The retention indices of the n-alkanes were used as standards. The starting temperatures and temperature programme rates used for the analysis are described in Section 2.9.1. The mean retention times for the DCBEs and n-alkanes are shown in Table 3.10, using a CPSil5-CB column and in Table 3.12, using a CPSil8-CB column. The retention times in full are given in Appendix 2, Tables A1-3 and A4-6 respectively.

The retention indices are shown in Table 3.11, using a CPSil5-CB column, and Table 3.13, using a CPSil8-CB column. The retention indices in full are given in Appendix 2, Tables A 7 and A 8 respectively.

It must be noted that in Table A5 in Appendix 2, in which a CPSil8-CB column and a temperature programme rate of 5 deg min^{-1} was used, the retention times of the DCBEs and alkanes do not change for the different starting temperatures. This does not correspond to the other observations, and suggests the gas chromatograph had not been reset, probably due to either a computer fault or a system communication problem. A problem was also encountered in calculating retention indices for the results recorded using a CPSil5-CB column, a starting temperature of 100°C and a programme rate of 5 deg min^{-1} as shown in Table A2 in Appendix 2. Only two sets of retention index data could be generated as a result of a computer communication fault.

Table 3.10 Mean Retention Times of DCBEs and n-Alkanes Using a CPSIL5-CB Column, Three Different Starting Temperatures and Temperature Programme Rates.

	GC Conditions		
	3 degmin^{-1}		
Compound	80°C	100°C	120°C
	Time (sec)		
C11	175.1 (2.90)	118.6 (0.62)	
C12	265.4 (2.48)	159.0 (2.21)	
C13	395.9 (2.30)	226.8 (2.13)	139.7 (0.33)
D2	497.6 (2.12)	291.7 (1.84)	173.8 (0.41)
C14	565.3 (2.03)	328.0 (1.70)	187.9 (0.42)
D3	670.5 (1.87)	405.3 (1.48)	232.9 (0.59)
D4	871.6 (1.88)	555.5 (1.26)	321.3 (0.82)
C16	962.1 (1.34)	623.9 (0.89)	359.5 (0.96)
D5	1078.7 (1.88)	728.6 (1.15)	437.6 (1.16)
D6	1285.0 (1.88)	913.7 (0.95)	577.8 (1.47)
C18	1368.6 (1.31)	988.4 (0.57)	634.0 (1.56)
D7	1487.9 (1.93)	1104.6 (0.96)	737.5 (1.68)
D8	1683.8 (1.76)	1292.5 (2.82)	906.3 (1.93)
C20	1752.1 (1.76)	1359.2 (0.48)	964.5 (1.96)
D9	1873.8 (2.07)	1480.2 (0.39)	1080.0 (2.07)
D10	2056.1 (2.07)	1660.3 (0.68)	1252.8 (1.83)
C22	2107.3 (1.81)	1710.3 (0.54)	1300.7 (2.21)
D11	2232.8 (2.13)	1835.6 (0.66)	1424.3 (2.25)
D12	2402.5 (2.41)	2004.7 (0.71)	1591.3 (2.17)
C24	2436.9 (0.73)	2038.4 (0.42)	1624.1 (2.79)
D13	2566.3 (2.08)	2168.4 (0.64)	1753.4 (2.52)
D14	2724.3 (2.09)	2326.2 (0.63)	1910.8 (2.28)
C26	2737.7 (1.19)	2343.0 (0.05)	1927.1 (2.42)
D15	2876.2 (1.76)	2478.1 (0.52)	2061.9 (2.37)
C28			
D16	3023.8 (2.25)	2626.3 (0.53)	2209.6 (2.23)
C30	3293.1 (1.55)	2895.5 (0.25)	2479.3 (2.43)
No. Injections	3	4	10

Standard deviations given in parentheses.

Table 3.10 (contd)

	GC Conditions		
	5 degmin^{-1}		
	80°C	100°C	120°C
	Time (sec)		
Compound			
C11	172.3 (0.30)	116.8 (0.10)	
C12	245.7 (0.40)	155.0 (0.10)	111.5 (0.15)
C13	344.5 (0.35)	213.7 (0.05)	140.9 (0.10)
D2	418.7 (0.30)	266.5 (0.00)	173.0 (0.10)
C14	462.8 (0.00)	293.7 (0.05)	185.5 (0.10)
D3	535.3 (0.30)	352.2 (0.10)	225.6 (0.05)
D4	665.2 (0.40)	458.4 (0.05)	298.1 (0.05)
C16	717.6 (0.25)	502.1 (0.05)	326.4 (0.10)
D5	795.6 (0.15)	574.4 (0.05)	386.4 (0.10)
D6	923.5 (0.00)	693.4 (0.00)	486.1 (0.05)
C18	969.7 (0.05)	736.5 (0.15)	521.2 (0.05)
D7	1048.8 (0.00)	813.9 (0.10)	593.5 (0.05)
D8	1168.5 (0.00)	931.0 (0.15)	702.8 (0.00)
C20	1204.4 (0.05)	966.4 (0.10)	735.1 (0.10)
D9	1284.5 (0.10)	1045.1 (0.45)	812.7 (0.25)
D10	1395.7 (0.25)	1156.1 (0.15)	920.6 (0.20)
C22	1421.5 (0.10)	1181.6 (0.10)	946.2 (0.65)
D11	1503.3 (0.10)	1263.2 (0.10)	1026.2 (0.30)
D12	1607.1 (0.25)	1366.6 (0.20)	1128.7 (0.35)
C24	1621.7 (0.21)	1381.2 (0.10)	1143.0 (0.20)
D13	1707.1 (0.10)	1466.0 (0.10)	1227.7 (0.20)
D14	1802.2 (0.35)	1561.9 (0.20)	1324.0 (0.55)
C26	1806.9 (0.25)	1565.6 (1.20)	1328.0 (0.45)
D15	1894.9 (0.15)	1654.5 (0.20)	1415.6 (0.40)
C28			
D16	1984.3 (0.35)	1742.1 (1.95)	1504.8 (0.25)
C30	b	1906.2 (0.20)	1663.6 (0.65
No. injections	2	8	2

b peak not recognised by data acquisition program

Table 3.10 (Contd)

Compound	GC Conditions: 7 degmin^{-1}		
	80°C	100°C	120°C
	Time (sec)		
C11	164.9 (0.55)	114.8 (0.17)	
C12	227.8 (0.55)	150.0 (0.12)	110.4 (0.42)
C13	308.1 (0.65)	201.5 (0.19)	137.7 (0.37)
D2	367.5 (0.75)	246.6 (0.26)	168.4 (0.68)
C14	399.3 (0.80)	268.5 (0.75)	178.5 (0.59)
D3	455.9 (0.80)	315.8 (0.24)	213.1 (0.50)
D4	552.5 (0.85)	398.9 (0.28)	273.1 (1.19)
C16	588.8 (0.80)	430.2 (0.24)	296.0 (0.12)
D5	649.4 (0.10)	486.7 (0.09)	345.3 (0.08)
D6	741.5 (0.75)	575.1 (0.09)	422.6 (0.41)
C18	772.4 (0.75)	604.6 (0.19)	447.3 (0.37)
D7	832.3 (0.80)	663.3 (0.24)	503.4 (0.62)
D8	918.4 (0.40)	748.6 (0.14)	584.6 (0.74)
C20	942.4 (0.70)	771.4 (0.17)	605.9 (0.62)
D9	1002.7 (0.85)	831.6 (0.24)	665.4 (0.83)
D10	1083.2 (0.80)	911.6 (0.14)	743.4 (0.38)
C22	1098.7 (0.60)	927.2 (0.12)	758.9 (0.74)
D11	1161.0 (0.80)	989.1 (0.25)	820.7 (0.91)
D12	1234.6 (0.90)	1063.6 (0.17)	894.6 (0.95)
C24	1243.5 (0.90)	1071.7 (0.08)	902.5 (0.86)
D13	1307.4 (0.95)	1135.4 (0.17)	965.9 (0.50)
D14			
C26	1376.9 (0.70)	1205.4 (0.33)	1035.8 (1.00)
D15	1443.2 (0.70)	1271.0 (0.00)	1102.1 (1.00)
C28	1502.8 (0.65)	1331.1 (0.09)	1161.7 (1.05)
D16	1508.3 (0.60)	1336.3 (0.25)	1167.0 (1.02)
C30	1617.4 (2.45)	1468.1 (0.12)	1278.7 (1.02)
no. Injections	2	3	2

Table 3.11 Mean Retention Indices of DCBEs Using a CPSIL5-CB Column, Three Different Starting Temperatures and Programme Rates.

	GC Conditions		
	3 degmin^{-1}		
	80°C	100°C	120°C
D2	1363 (0.47)	1368 (1.00)	1373 (0.46)
D3	1455 (0.47)	1461 (1.78)	1473 (0.30)
D4	1556 (0.00)	1560 (1.00)	1569 (0.30)
D5	1651 (0.00)	1659 (0.43)	1660 (0.49)
D6	1758 (0.47)	1760 (0.43)	1762 (0.00)
D7	1861 (0.47)	1862 (0.00)	1866 (0.00)
D8	1963 (0.00)	1965 (1.00)	1966 (0.00)
D9	2067 (0.47)	2067 (0.00)	2068 (0.00)
D10	2170 (0.47)	2171 (0.43)	2171 (0.00)
D11	2275 (0.47)	2275 (0.43)	2275 (0.00)
D12	2379 (0.82)	2379 (0.00)	2379 (0.00)
D13	2483 (0.47)	2483 (0.43)	2483 (0.40)
D14	2589 (0.47)	2588 (0.43)	2588 (0.49)
D15	2694 (0.47)	2694 (0.43)	2694 (0.30)
D16	2800 (0.00)	2800 (0.00)	2800 (0.00)
		5degmin^{-1}	
D2	1365 (0.00)	1370 (0.00)	1373 (0.00)
D3	1458 (0.00)	1461 (0.00)	1472 (0.00)
D4	1559 (0.00)	1561 (0.00)	1568 (0.50)
D5	1661 (0.00)	1662 (0.00)	1664 (0.00)
D6	1763 (0.00)	1764 (0.00)	1765 (0.50)
D7	1865 (0.00)	1866 (0.00)	1868 (0.00)
D8	1968 (0.00)	1968 (0.00)	1969 (0.50)
D9	2072 (0.00)	2072 (0.00)	2073 (0.00)
D10	2175 (0.50)	2175 (0.00)	2177 (0.00)
D11	2280 (0.00)	2279 (0.00)	2280 (0.50)
D12	2384 (0.50)	2384 (0.00)	2384 (0.50)
D13	2490 (0.00)	2489 (0.00)	2490 (0.50)
D14	2594 (0.00)	2594 (0.00)	2594 (0.50)
D15	2698 (0.00)	2699 (0.00)	2698 (1.00)
D16	2800 (0.00)	2800 (0.00)	2802 (2.50)

Table 3.11 (contd)

	GC Conditions 7 degmin^{-1}		
	80°C	100°C	120°C
D2	1366 (0.00)	1371 (0.47)	1375 (1.00)
D3	1460 (0.00)	1463 (0.82)	1473 (1.00)
D4	1562 (0.00)	1564 (0.47)	1569 (0.50)
D5	1663 (0.50)	1665 (0.47)	1667 (1.00)
D6	1765 (0.00)	1766 (0.66)	1769 (0.00)
D7	1869 (0.00)	1869 (0.00)	1871 (0.50)
D8	1972 (0.00)	1972 (0.00)	1972 (0.50)
D9	2075 (0.00)	2075 (0.00)	2076 (0.50)
D10	2179 (0.00)	2179 (0.00)	2180 (0.50)
D11	2284 (0.00)	2284 (0.47)	2283 (0.50)
D12	2389 (1.00)	2388 (0.00)	2388 (0.00)
D13	2494 (0.00)	2494 (0.47)	2494 (0.47)
D14	2600 (0.00)	2600 (0.00)	2600 (0.00)
D15	2702 (0.00)	2703 (0.47)	2704 (1.00)
D16	2802 (0.00)	2806 (1.70)	2809 (1.00)

Table 3.12 Mean Retention Times of DCBEs and n-Alkanes Using a CPSIL8-CB Column, Three Different Starting Temperatures and Temperature Programme Rates.

	GC Conditions 3 degmin^{-1}		
	80°C	100°C	120°C
Compound	Time (sec)		
C12	337.6 (2.03)	206.2 (0.78)	
C13	492.4 (1.88)	293.2 (0.78)	187.0 (0.30)
D2	645.8 (1.73)	399.0 (0.71)	
C14	681.8 (1.65)	417.0 (0.71)	253.1 (0.35)
D3	837.1 (1.51)	538.7 (0.86)	332.1 (0.30)
D4	1051.4 (1.29)	714.0 (0.86)	450.2 (0.35)
C16	1106.1 (1.80)	752.2 (0.88)	471.2 (0.40)
D5	1268.4 (1.64)	906.4 (0.94)	596.1 (0.40)
D6	1480.5 (1.57)	1104.3 (1.11)	761.1 (0.45)
C18	1520.8 (1.57)	1139.9 (1.19)	787.0 (0.56)
D7	1689.0 (1.75)	1304.2 (1.23)	939.2 (0.50)
D8	1887.9 (1.96)	1498.8 (1.49)	1120.9 (0.50)
C20	1913.4 (1.87)	1522.6 (1.39)	1140.7 (0.45)
D9	2081.6 (1.90)	1689.7 (1.56)	1303.9 (0.45)
D10	2267.0 (2.03)	1873.8 (1.88)	1483.6 (0.30)
C22	2276.0 (1.99)	1882.4 (1.68)	1490.7 (0.25)
D11	2445.9 (2.32)	2052.3 (1.73)	1659.2 (0.05)
C24	2611.7 (2.04)	2217.6 (1.48)	1822.3 (0.30)
D12	2618.7 (1.99)	2224.9 (1.65)	1830.0 (0.25)
D13	2784.2 (2.17)	2390.6 (1.43)	1994.7 (0.20)
C26	2922.0 (2.12)	2528.5 (1.44)	2131.8 (0.25)
D14	2944.4 (2.22)	2550.9 (1.42)	2154.5 (0.15)
D15	3098.3 (2.27)	2705.1 (1.39)	2308.5 (0.20)
C28	3213.2 (2.36)	2820.0 (1.30)	2423.5 (0.05)
D16	3248.1 (2.48)	2855.2 (1.16)	2458.5 (0.05)
C30	3484.1 (2.53)	3091.7 (1.05)	2694.6 (0.25)
No. injections	11	3	2

Table 3.12 (contd)

GC Conditions

5 degmin^{-1}

Compound	80°C	100°C	120°C
	Time (sec)		
C13			180.7 (0.17)
D2			
C14	237.7 (0.12)	237.5 (0.53)	237.1 (0.22)
D3	303.1 (0.41)	302.8 (0.37)	302.1 (0.25)
D4	392.9 (0.75)	392.6 (0.41)	391.8 (0.33)
C16	405.6 (0.71)	405.3 (0.37)	404.4 (0.29)
D5	496.6 (0.80)	496.1 (0.59)	495.1 (0.33)
D6	608.0 (0.91)	607.3 (0.89)	606.3 (0.38)
C18	620.7 (0.93)	620.1 (0.83)	619.1 (0.30)
D7	723.4 (1.02)	722.8 (1.29)	721.4 (0.41)
D8	838.6 (1.08)	837.8 (1.13)	836.1 (0.43)
C20	845.7 (1.11)	844.9 (1.30)	843.3 (0.39)
D9	952.6 (1.37)	951.8 (1.50)	950.1 (0.56)
D10			
C22	1062.9 (1.71)	1062.3 (1.57)	1060.5 (0.46)
D11	1171.3 (1.71)	1170.8 (1.75)	1168.7 (0.59)
C24	1265.1 (1.62)	1264.6 (1.68)	1262.4 (0.35)
D12	1275.6 (1.75)	1275.1 (1.64)	1273.6 (1.35)
D13	1376.5 (0.88)	1375.1 (2.12)	1373.5 (0.43)
C26	1454.0 (1.84)	1453.6 (1.75)	1451.5 (0.32)
D14	1473.7 (1.85)	1473.3 (1.75)	1471.0 (0.35)
D15	1567.2 (1.79)	1566.9 (1.82)	1564.9 (0.53)
C28	1631.1 (1.87)	1631.0 (1.61)	1628.6 (0.31)
D16	1656.4 (4.32)	1658.3 (1.79)	1655.7 (0.35)
C30	1796.3 (2.00)	1796.2 (1.78)	1793.8 (0.31)
No. injections	3	4	8

Table 3.12 (contd)

GC Conditions

7 degmin^{-1}

Compound	80°C	100°C	120°C
	Time (sec)		
C12	276.5 (0.65)	188.2 (0.28)	139.8 (0.60)
C13	366.6 (0.95)	249.1 (0.30)	174.9 (0.60)
D2	451.0 (0.50)	317.1 (0.90)	
C14	464.1 (1.05)	324.5 (0.35)	223.7 (0.65)
D3	564.2 (0.15)	395.4 (1.08)	279.0 (0.90)
D4	646.1 (1.20)	486.3 (0.37)	350.7 (0.90)
C16	662.5 (1.25)	500.0 (0.37)	359.3 (0.85)
D5	745.1 (1.25)	579.5 (0.34)	430.9 (1.00)
D6	840.3 (1.20)	671.5 (0.41)	514.7 (1.05)
C18	850.7 (1.40)	681.2 (0.39)	522.0 (1.10)
D7	932.8 (1.20)	761.1 (0.41)	600.2 (1.00)
D8		849.3 (0.38)	686.1 (2.25)
C20	1020.3 (0.40)	852.5[a] (0.40)	
D9	1106.3 (1.15)	934.2 (0.43)	767.4 (0.65)
D10			
C22	1188.2 (1.05)	1016.1 (0.37)	847.6 (0.55)
D11	1267.0 (1.10)	1094.3 (0.55)	925.2 (0.00)
C24	1332.0 (1.05)	1159.4 (0.40)	990.1 (0.30)
D12	1342.8 (1.05)	1169.9 (0.45)	1000.8 (0.30)
D13	1415.8 (1.10)	1242.5 (0.42)	1073.1 (0.20)
C26	1469.1 (0.85)	1296.1 (0.39)	1126.3 (0.00)
D14	1486.1 (0.90)	1313.1 (0.49)	1143.2 (0.15)
D15	1553.8 (0.90)	1380.7 (0.48)	1210.9 (0.00)
C28	1597.3 (0.45)	1423.9 (0.55)	1254.1 (0.05)
D16	1619.5 (0.85)	1446.4 (0.40)	1276.6 (0.10)
C30	1716.0 (0.95)	1543.1 (0.48)	1373.3 (0.05)
No. injections	3	4	2

a 2 replicates

Table 3.13 Mean Retention Indices Of DCBEs Using a CPSIL8-CB Column , Three Different Starting Temperatures and Programme Rates.

	GC Conditions 3 degmin^{-1}		
	80°C	100°C	120°C
D2	1382 (0.00)	1388 (0.00)	1400 (0.00)
D3	1475 (0.00)	1479 (0.00)	1485 (0.00)
D4	1576 (0.44)	1579 (0.00)	1584 (0.50)
D5	1679 (0.00)	1681 (0.00)	1685 (0.00)
D6	1780 (0.00)	1782 (0.00)	1785 (0.00)
D7	1884 (0.00)	1885 (0.00)	1887 (0.00)
D8	1987 (0.00)	1987 (0.00)	1989 (0.00)
D9	2091 (0.00)	2091 (0.00)	2092 (0.00)
D10	2195 (0.00)	2195 (0.00)	2196 (0.00)
D11	2299 (0.00)	2299 (0.00)	2300 (0.00)
D12	2404 (0.00)	2405 (0.00)	2405 (0.00)
D13	2509 (0.39)	2510 (0.00)	2510 (0.00)
D14	2615 (0.00)	2615 (0.00)	2615 (0.00)
D15	2719 (0.00)	2719 (0.00)	2719 (0.00)
D16	2825 (0.00)	2825 (0.00)	2825 (0.00)
	5degmin^{-1}		
D2	1400 (0.00)	1400 (0.00)	1400 (0.00)
D3	1487 (0.00)	1487 (0.00)	1487 (0.00)
D4	1587 (0.00)	1587 (0.00)	1587 (0.00)
D5	1688 (0.00)	1688 (0.00)	1688 (0.00)
D6	1789 (0.00)	1789 (0.00)	1789 (0.00)
D7	1891 (0.00)	1891 (0.00)	1891 (0.00)
D8	1994 (0.00)	1994 (0.00)	1994 (0.00)
D9	2097 (0.00)	2097 (0.00)	2097 (0.00)
D10	2200 (0.00)	2200 (0.00)	2200 (0.00)
D11	2305 (0.47)	2305 (0.47)	2305 (0.00)
D12	2411 (0.00)	2411 (0.00)	2411 (0.00)
D13	2516 (0.00)	2516 (0.00)	2516 (0.00)
D14	2622 (0.00)	2622 (0.00)	2622 (0.00)
D15	2726 (0.00)	2726 (0.00)	2726 (0.00)
D16	2832 (0.00)	2832 (0.00)	2832 (0.00)

Table 3.13 (contd)

	GC Conditions 7 degmin^{-1}		
	80°C	100°C	120°C
D2	1391 (0.00)	1391 (0.35)	1400 (0.00)
D3	1481 (0.00)	1484 (0.00)	1489 (0.00)
D4	1583 (0.00)	1585 (0.00)	1589 (0.00)
D5	1685 (0.00)	1687 (0.83)	1690 (0.00)
D6	1788 (0.00)	1789 (0.00)	1791 (0.00)
D7	1894 (0.50)	1895 (1.16)	1896 (0.00)
D8	1998 (1.15)	2000 (0.00)	2000 (0.00)
D9	2100 (0.00)	2100 (0.00)	2099 (0.50)
D10	2200 (0.00)	2200 (0.00)	2200 (0.00)
D11	2307 (0.00)	2307 (0.00)	2307 (0.00)
D12	2415 (0.00)	2415 (0.00)	2415 (0.50)
D13	2521 (0.00)	2520 (0.49)	2521 (0.00)
D14	2626 (0.00)	2626 (0.00)	2626 (0.00)
D15	2731 (0.00)	2730 (0.00)	2730 (0.50)
D16	2837 (0.00)	2837 (0.49)	2837 (0.00)

3.5 RETENTION INDICES OF ORGANOCHLORINE STANDARD COMPOUNDS

3.5.1 Retention Indices of Organochlorine Standard Compounds on a CPSil5-CB Column Using Different Starting Temperatures and Temperature Programme Rates

Using the DCBE retention index values from Section 3.4, retention indices were calculated for organochlorine compounds in the two standard solutions as described in Section 2.9.2. Retention indices were calculated for the DCBE standard solutions on a CPSil5-CB column using two temperature programme rates and three starting temperatures. Different DCBEs were chosen as internal standards (D7, D12 and D14) and retention indices were also calculated using selected DCBEs as calibration standards, i.e. D4, D6, D7, D8, D10, D12, D14 and D16. Results are shown in Table 3.14a for a starting temperature of 80°C and a programme rate of 3 deg min^{-1} for two injections (A and B). Tables 3.14b and 3.14c show results calculated for single DCBE injections using starting temperatures of 100°C and 120°C respectively. Only one injection was made at these conditions. In all cases two injections were made at each set of conditions for each solution. However, because the chromatogram was complex, the data acquisition programme ('Chromatochart') could not always recognise all individual peaks although they may be resolved. As a result not all injections generated usable results and thus for some sets of conditions only one data set is given. This will be discussed further in Chapter 4.

The retention indices of the organochlorines using a CPSil5-CB column using the different starting temperatures are shown in Table 3.15. In each case the value given is that calculated using D7 as an internal standard and the value in parentheses is the maximum deviation observed from that value. Where no value is given in parenthesis the

Table 3.14a Retention indices of DCBEs using a CPSil5-CB column using a programme rate of 3 deg min^{-1}, a starting temperature of 80°C and different DCBEs as internal standards and calibration standards.

Injection A		Compound Treated as Internal Standard							
		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	586.5	1363	1365	1363	1365	1363	1363	1363	1363
D3	772.3	1455	1456	1455	1456	1455	1454	1455	1454
D4	979.8	1555	1556	1556	1556	1556	1556	1556	1556
D5	1188.8	1657	1656	1656	1656	1657	1656	1657	1656
D6	1396.7	1758	1758	1757	1757	1757	1757	1758	1758
D7	1522.3	1861	1861	1860	1860	1860	1860	1861	1860
D8	1798.6	1963	1963	1963	1963	1963	1963	1963	1962
D9	1989.5	2067	2067	2066	2066	2066	2066	2067	2066
D10	2173.2	2170	2170	2170	2170	2170	2170	2170	2170
D11	2351.0	2275	2274	2275	2274	2274	2274	2275	2274
D12	2522.5	2379	2379	2379	2379	2379	2378	2379	2379
D13	2688.3	2483	2483	2483	2483	2483	2483	2483	2483
D14	2747.8	2588	2589	2589	2589	2589	2589	2589	2589
D15	3000.3	2694	2691	2694	2694	2694	2694	2694	2694
D16	3148.4	2800	2799	2800	2800	2800	2799	2800	2800

Table 3.14a (contd.)

Injection B

Ether	Time (sec)	Compound Treated as Internal Standard: No Int. Std. Complete Series	No Int. Std. Incomplete Series	D7 Complete Series	D7 Incomplete Series	D12 Complete Series	D12 Incomplete Series	D14 Complete Series	D14 Incomplete Series
D2	583.7	1361	1361	1361	1361	1361	1361	1361	1361
D3	769.5	1453	1453	1453	1454	1453	1453	1453	1453
D4	977.1	1554	1554	1554	1554	1554	1554	1554	1554
D5	1186.1	1655	1655	1655	1655	1655	1655	1655	1655
D6	1394.7	1757	1757	1756	1756	1756	1756	1757	1757
D7	1602.4	1861	1861	1860	1860	1861	1860	1861	1861
D8	1798.8	1963	1963	1962	1962	1962	1962	1963	1963
D9	1989.3	2066	2066	2065	2064	2066	2066	2067	2066
D10	2175.0	2171	2171	2169	2169	2170	2170	2171	2171
D11	2352.0	2275	2275	2274	2273	2275	2274	2275	2275
D12	2522.9	2379	2379	2379	2379	2379	2379	2379	2379
D13	2687.9	2483	2483	2481	2481	2482	2483	2483	2483
D14	2847.3	2588	2588	2586	2586	2588	2588	2589	2589
D15	2999.8	2693	2690	2691	2692	2693	2692	2694	2694
D16	3148.0	2799	2799	2797	2797	2799	2799	2800	2800

Table 3.14b Retention indices of DCBEs using a CPSil5-CB column, a programme rate of 3 deg min^{-1}, starting temperature of 100°C and different DCBEs as internal standards and calibration standards.[a]

		Compound Treated as Internal Standard							
		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	373.4	1367	1401	1367	1401	1366	1401	1367	1401
D3	507.5	1461	1471	1461	1471	1460	1471	1461	1471
D4	675.4	1559	1560	1560	1560	1560	1560	1560	1560
D5	862.3	1658	1658	1659	1658	1659	1658	1659	1658
D6	1057.3	1760	1760	1759	1760	1760	1759	1760	1760
D7	1255.6	1862	1862	1861	1861	1862	1862	1862	1862
D8	1449.6	1964	1964	1964	1964	1963	1963	1963	1963
D9	1639.7	2067	2067	2066	2067	2067	2067	2066	2067
D10	1824.1	2171	2171	2171	2171	2171	2171	2171	2170
D11	2003.6	2275	2275	2275	2275	2274	2275	2274	2275
D12	2176.5	2379	2379	2378	2379	2379	2379	2379	2379
D13	2343.8	2483	2483	2482	2483	2483	2483	2483	2483
D14	2504.6	2588	2587	2587	2587	2588	2588	2588	2588
D15	2659.4	2694	2691	2693	2689	2694	2692	2694	2694
D16	2809.7	2799	2800	2799	2800	2800	2800	2799	2799

a. Only one injection was made using these conditions.

Table 3.14c Retention indices of DCBEs using a CPSil5-CB column, a programme rate of 3 deg min^{-1}, a starting temperature of 120°C and different DCBEs as internal standards and calibration standards.

		Compound Treated as Internal Standard							
		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	237.5	1373	1445	1373	1445	1373	1445	1373	1445
D3	306.1	1473	1496	1473	1496	1473	1496	1473	1496
D4	418.3	1569	1569	1569	1568	1569	1568	1569	1569
D5	858.7	1660	1660	1659	1660	1659	1660	1660	1660
D6	720.6	1761	1761	1762	1761	1762	1762	1762	1762
D7	897.1	1866	1866	1866	1866	1866	1866	1866	1866
D8	1079.6	1965	1966	1966	1966	1966	1966	1966	1966
D9	1262.8	2067	2067	2068	2067	2068	2067	2068	2067
D10	1444.5	2171	2171	2171	2170	2171	2171	2171	2171
D11	1622.5	2275	2275	2274	2275	2274	2275	2274	2275
D12	1795.3	2379	2378	2379	2379	2379	2378	2378	2378
D13	1962.8	2483	2483	2483	2483	2483	2483	2483	2483
D14	2124.4	2588	2588	2588	2588	2588	2588	2588	2588
D15	2279.9	2693	2690	2694	2694	2694	2692	2694	2693
D16	2431.1	2799	2800	2800	2799	2800	2800	2799	2800

Table 3.15 Retention indices of organochlorine compounds using a CPSil5-CB column, a temperature programme of 3 deg min^{-1} and different starting temperatures.[a]

	Starting temp. 80°C		Starting temp. 100°C		Starting temp. 120°C	
HCB	1649 (+1)	1649 (-1)	1655 (±1)	1655 (-1)	1662	1662
PCB28	1807 (-1)	1807 (-1)	1810 (+1)	1810	1816	1816
Hept	1832 (+1)	1833 (-1)	1836 (+1)	1836	1844	1844 (-1)
D7	1860 (-1)	1860 (+1)	1861 (+2)	1861 (+1)	1866	1866 (-1)
PCB52	1877 (-1)	1877 (-1)	1879 (+2)	1879	1885	1885 (-1)
Aldrin	1897 (-1)	1897 (-1)	1900 (+2)	1899 (+2)	1907	1907
PCB44	1910	1910	1913 (+2)	1913 (-1)	1918	1918 (-1)
2,4 DDE	2042 (-1)	2041 (+1)	2042 (+3)	2043 (+1)	2044 (+1)	2045 (-1)
PCB101	2048 (-1)	2048 (-1)	2049 (+3)	2050 (+1)	2051	2051
4,4 DDE	2108 (-1)	2107	2108 (+2)	2109 (+1)	2109 (+1)	2110 (-1)
PCB118	2168 (-1)	2168	2169 (+1)	2170 (+1)	2170	2170
PCB153	2223 (-1)	2222 (+1)	2223 (+1)	2233 (+2)	2223 (+1)	2224 (-1)
PCB137	2255	2255	2254 (+2)	2256 (+1)	2256	2256
PCB138	2271 (-1)	2271	2269 (+2)	2271 (+1)	2271	2272 (-1)
PCB128	2319 (-1)	2318 (-1)	2317 (+2)	2318 (+2)	2318 (+1)	2319 (-1)
PCB180	2421 (-1)	2420 (+1)	2419 (+2)	2421 (+2)	2421	2422 (-1)
Mirex	2448 (-1)	2447 (+1)	2446 (+3)	2449 (+1)	2449 (+1)	2450 (-1)
PCB195	2573 (-1)	2572 (+1)	2570 (+2)	2571 (+1)	2572	2573 (-1)
D14	2589 (-1)	2588 (+1)	2585 (+4)	2587 (+2)	2587	2588 (-1)
PCB194	2626 (-2)	2625 (+1)	2622 (+5)	2622 (+5)	2624 (-2)	2626 (-3)

Solution S1

Programme rate 3 deg min^{-1}

a See Section 3.5.1 for details of calculations.

Table 3.15 (contd.)

	Starting temp. 80°C		100°C	Starting temp. 120°C	
α HCH	1622 (-1)	1622	1628 (-1)	1636	1635 (-1)
β HCH	1650 (-1)	1650 (-1)	1655 (-1)	1661	1661
γ HCH	1679	1679	1683 (+1)	1689 (+1)	1689 (-1)
Heptachlor	1833 (-1)	1833 (-1)	1836	1844	1844
α Chordene	1843 (-1)	1843 (+1)	1846 (+1)	1854	1853
D7	1860 (-1)	1860 (-1)	1861 (+1)	1866	1865
γ Chlordene	1892 (-1)	1892 (-1)	1894 (+1)	1901 (+1)	1901
Hept Epox	1968	1968 (-1)	1971 (+1)	1975 (-1)	1975
Oxychloridane	1975	1976 (-1)	1978 (+1)	1982 (+1)	1982
γ Chlordane	2012 (-1)	2012 (-1)	2014 (+1)	2017 (+1)	2017
Endosulphanl	2036 (-2)	2036 (-2)	2037 (+1)	2041 (-1)	2041 (-1)
2,4 DDE	2042 (-1)	2042 (-1)	2043 (+1)	2045 (-1)	2045 (-1)
α Chlordane	2046 (-1)	2046 (-1)	2048 (+1)	2051 (-1)	2051 (-1)
tr-nonachlor	2065 (-2)	2065 (-2)	2066 (+1)	2069	2069 (-1)
Dieldrin	2087 (-2)	2087 (-1)	2088 (+1)	2091 (-1)	2091 (-1)
2,4 DDD	2113 (-1)	2113 (-1)	2115 (+1)	2116 (-1)	2116 (-1)
Endrin	2119 (-1)	2119 (-1)	2121 (+1)	2123 (+1)	2124 (-1)
4,4 DDT	2179 (-1)	2179 (-1)	2180 (+1)	2181	2181
2,4 DDT	2193 (-1)	2193 (-1)	2194 (+1)	2194 (+1)	2195 (-1)
4,4 DDT	2264 (-1)	2264 (-1)	2263 (+1)	2264 (+1)	2265 (-1)
D12	2379 (-1)	2380 (-2)	2377 (+2)	2378 (+1)	2379 (-1)
PADS	2469 (-1)	2469 (-1)	2467 (+2)	2468 (+1)	2469
cis perm	2618 (-1)	2619 (-2)	2615 (+2)	2615 (+1)	2617 (-2)
tr perm	2636 (-2)	2636 (-2)	2633 (-3)	2631 (+1)	2635 (-4)

Solution S2

Programme rate 3 deg min^{-1}

a See Section 3.5.1 for details of calculations.

values were identical. The retention indices of the DCBEs calculated at a temperature programme rate of 5 deg min^{-1} are shown in Table 3.16a-c for three starting temperatures. The corresponding organochlorine values are shown in Tables 3.17

3.5.2 Retention Indices of Organochlorine Standard Compounds on a CPSil8-CB Column Using Different Starting Temperatures and Temperature Programme Rates

Retention indices were calculated for the DCBEs and organochlorines as previously described above. Results for the DCBEs are shown in Table 3.18a-3.18c the DCBEs using three different starting temperatures and a temperature programme rate of 3 deg min^{-1} and the organochlorine values are shown in Table 3.19. The DCBE values recorded using a programme rate of 5 deg min^{-1} are shown in Tables 3.20a-3.20c. The organochlorine values are shown in Table 3.21.

Table 3.16a Retention indices of DCBEs using a CPSil5-CB column, using a programme rate of 5 deg min^{-1}, a starting temperature of 80°C and different DCBEs as internal standards and calibration standards.

		Injection A							
		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	513.2	1365	1363	1364	1363	1364	1363	1364	1363
D3	633.3	1458	1457	1458	1457	1457	1457	1457	1457
D4	765.8	1558	1559	1559	1559	1559	1559	1558	1559
D5	898.0	1661	1660	1661	1660	1661	1660	1661	1660
D6	1028.4	1763	1763	1763	1763	1762	1763	1762	1763
D7	1152.1	1865	1865	1865	1865	1864	1865	1865	1865
D8	1274.0	1968	1968	1968	1968	1968	1967	1967	1967
D9	1391.5	2071	2071	2072	2071	2072	2071	2072	2071
D10	1504.8	2175	2175	2175	2174	2174	2175	2175	2174
D11	1613.5	2280	2278	2279	2278	2280	2278	2278	2278
D12	1719.1	2384	2384	2384	2384	2383	2383	2383	2383
D13	1819.5	2490	2489	2490	2489	2490	2489	2489	2489
D14	1916.3	2594	2594	2594	2593	2594	2594	2593	2594
D15	2010.8	2698	2696	2697	2697	2698	2698	2698	2698
D16	2103.3	2800	2800	2799	2799	2800	2799	2800	2800

Table 3.16a (contd.)

Injection B

Ether	Time (sec)	No Int. Std. Complete Series	No Int. Std. Incomplete Series	D7 Complete Series	D7 Incomplete Series	D12 Complete Series	D12 Incomplete Series	D14 Complete Series	D14 Incomplete Series
D2	512.8	1364	1362	1364	1362	1364	1362	1364	1362
D3	632.5	1457	1456	1457	1456	1457	1456	1457	1456
D4	765.3	1558	1558	1558	1558	1558	1558	1558	1558
D5	897.6	1660	1659	1660	1659	1660	1659	1660	1659
D6	1028.5	1763	1763	1762	1762	1763	1763	1762	1762
D7	1152.8	1867	1865	1865	1865	1865	1865	1865	1865
D8	1274.4	1968	1968	1867	1967	1968	1967	1967	1967
D9	1392.0	2072	2070	2071	2070	2071	2071	2071	2071
D10	1505.0	2173	2175	2174	2174	2175	2175	2174	2174
D11	1614.0	2280	2279	2279	2278	2279	2279	2279	2278
D12	1719.1	2384	2384	2382	2382	2383	2383	2383	2383
D13	1819.7	2490	2489	2489	2488	2490	2489	2489	2488
D14	1917.1	2594	2594	2593	2593	2594	2594	2593	2594
D15	2010.7	2697	2696	2696	2696	2697	2698	2696	2697
D16	2103.4	2800	2799	2800	2800	2799	2800	2800	2800

Table 3.16b Retention indices of DCBEs using a CPSil5-CB column, a starting temperature of 100°C, a temperature programme rate of 5 deg min^{-1} and different DCBEs as internal standards and calibration standards.

Injection A

		No Int. Std.		D7		D12		D14	
Ether	Time (Sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	331.7	1370	1386	1369	1386	1369	1386	1369	1386
D3	427.0	1460	1465	1460	1465	1461	1465	1461	1465
D4	541.7	1561	1561	1561	1560	1560	1561	1561	1560
D5	662.6	1661	1661	1662	1661	1662	1661	1661	1661
D6	785.9	1764	1764	1763	1764	1764	1764	1764	1764
D7	908.1	1866	1866	1865	1866	1866	1866	1865	1865
D8	1027.2	1968	1968	1968	1968	1867	1967	1967	1968
D9	1143.8	2072	2072	2072	2072	2072	2072	2072	2072
D10	1255.7	2176	2176	2176	2175	2175	2175	2175	2176
D11	1366.2	2280	2280	2280	2280	2280	2280	2280	2280
D12	1469.4	2384	2385	2385	2385	2385	2385	2384	2385
D13	1569.7	2490	2489	2490	2489	2490	2489	2490	2489
D14	1666.7	2594	2593	2594	2594	2594	2594	2594	2594
D15	1760.4	2698	2697	2698	2696	2698	2697	2698	2697
D16	1850.4	2800	2800	2800	2800	2800	2800	2799	2800

Table 3.16b (contd.)

Injection B

Ether	Time (sec)	No Int. Std.		D7		D12		D14	
		Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	331.4	1369	1385	1369	1386	1369	1386	1369	1386
D3	426.7	1460	1465	1460	1465	1460	1465	1460	1465
D4	541.7	1560	1560	1561	1561	1561	1560	1560	1560
D5	662.7	1661	1661	1662	1661	1661	1661	1661	1661
D6	786.2	1763	1763	1764	1764	1763	1763	1763	1763
D7	908.7	1865	1866	1866	1866	1866	1866	1866	1866
D8	1028.0	1968	1968	1968	1968	1968	1968	1968	1968
D9	1144.7	2072	2072	2072	2072	2072	2072	2072	2072
D10	1256.8	2176	2176	2177	2177	2176	2176	2176	2176
D11	1365.1	2279	2280	2280	2280	2280	2280	2280	2280
D12	1470.2	2384	2384	2385	2385	2385	2385	2385	2385
D13	1570.4	2489	2488	2490	2490	2489	2489	2489	2489
D14	1667.5	2593	2593	2594	2594	2593	2593	2594	2594
D15	1760.5	2697	2695	2698	2697	2697	2696	2697	2696
D16	1851.5	2799	2799	2800	2800	2800	2799	2799	2799

Table 3.16c Retention indices of DCBEs using a CPSil5-CB column, a starting temperature of 120°C, a temperature programme of 5 deg min^{-1} and different DCBEs as internal standards and calibration standards.

Injection A

		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	216.3	1373	1426	1372	1426	1372	1426	1373	1426
D3	250.8	1472	1487	1472	1483	1471	1487	1472	1487
D4	365.7	1569	1569	1569	1569	1568	1568	1569	1569
D5	464.0	1663	1663	1663	1663	1664	1663	1664	1663
D6	571.9	1765	1765	1764	1765	1765	1765	1765	1765
D7	684.4	1867	1868	1867	1867	1868	1868	1868	1868
D8	797.3	1970	1970	1969	1969	1969	1970	1969	1970
D9	910.0	2073	2073	2073	2073	2073	2073	2073	2073
D10	1017.8	2177	2177	2177	2177	2176	2177	2176	2177
D11	1126.8	2280	2280	2280	2280	2280	2280	2279	2280
D12	1230.9	2385	2385	2385	2385	2385	2385	2385	2385
D13	1330.8	2491	2488	2491	2488	2491	2488	2491	2488
D14	1428.9	2594	2594	2594	2594	2594	2594	2593	2594
D15	1521.6	2699	2697	2699	2695	2699	2696	2698	2696
D16	1611.7	2800	2800	2800	2800	2799	2800	2799	2799

Table 3.16c (contd.)

Injection B

Ether	Time (sec)	No Int. Std. Complete Series	No Int. Std. Incomplete Series	D7 Complete Series	D7 Incomplete Series	D12 Complete Series	D12 Incomplete Series	D14 Complete Series	D14 Incomplete Series
D2	216.2	1372	1426	1372	1425	1371	1425	1372	1425
D3	280.5	1471	1487	1471	1486	1470	1486	1471	1486
D4	365.6	1568	1568	1568	1568	1567	1568	1568	1568
D5	464.2	1664	1663	1663	1662	1663	1662	1663	1662
D6	572.5	1765	1765	1764	1764	1764	1764	1764	1764
D7	685.4	1868	1868	1867	1867	1867	1867	1868	1868
D8	799.0	1971	1971	1970	1970	1969	1969	1970	1970
D9	912.1	2074	2075	2073	2074	2072	2073	2073	2074
D10	1022.2	2179	2179	2177	2177	2176	2176	2177	2177
D11	1129.6	2282	2283	2281	2281	2279	2280	2281	2281
D12	1234.0	2388	2388	2386	2386	2385	2385	2386	2386
D13	1333.9	2494	2491	2492	2489	2490	2488	2492	2489
D14	1430.9	2596	2596	2593	2593	2592	2592	2593	2594
D15	1524.3	2702	2700	2699	2695	2697	2694	2699	2697
D16	1614.3	2800	2800	2800	2800	2798	2798	2800	2800

Table 3.17 Retention indices of organochlorine standard solutions using a CPSil5–CB column, three different starting temperatures and a temperature programme of 5 deg min^{-1}.

	G C Conditions					
	Starting temp. 80°C		Starting temp. 100°C		Starting temp. 120°C	
HCB	1660 (+2)	1661 (+1)	1663 (+1)	1663	1670 (+1)	1670 (+2)
PCB28	1817 (+3)	1817 (+2)	1818 (+1)	1818 (+1)	1822 (+1)	1822 (+2)
Hept	1846 (+3)	1846 (+2)	1847 (+1)	1847 (+1)	1852 (+1)	1852 (+3)
D7	1865 (+3)	1865 (+1)	1865 (+2)	1865 (+1)	1867 (+2)	1867 (+3)
PCB52	1886 (+3)	1886	1887 (+1)	1887 (+1)	1890 (+2)	1890 (+3)
Aldrin	1912 (+3)	1912 (+1)	1913 (+1)	1913 (+1)	1918 (+1)	1918 (+3)
PCB44	1921 (+3)	1921 (+1)	1922 (+1)	1922 (+1)	1926 (+1)	1925 (+3)
2,4 DDE	2053 (+4)	2052 (+2)	2052 (+2)	2053	2055 (+2)	2054 (+4)
PCB 101	2060 (+4)	2059 (+2)	2059 (+2)	2061 (-1)	2062 (+2)	2062 (+3)
4,4 DDE	2118 (+4)	2117 (+2)	2118 (+2)	2119	2121 (+2)	2120 (+4)
PCB 118	2181 (+4)	2180 (+2)	2182 (+2)	2182 (+1)	2184 (+2)	2184 (+4)
PCB 153	2235 (+5)	2234 (+2)	2237 (-2)	2236 (+1)	2237 (+3)	2236 (+5)
PCB 137	2269 (+5)	2268 (+3)	2269 (+2)	2270 (+1)	2271 (+3)	2270 (+5)
PCB 138	2284 (+3)	2283 (+3)	2284 (+2)	2285 (+1)	2286 (+3)	2285 (+5)
PCB 128	2333 (+5)	2332 (+2)	2333 (+3)	2334 (+1)	2335 (+3)	2334 (+5)
PCB 180	2435 (+6)	2435 (+3)	2437 (+3)	2438 (-2)	2440 (+2)	2438 (+6)
Mirex	2474 (+6)	2474 (+3)	2476 (+2)	2476 (+2)	2479 (+3)	2477 (+6)
PCB 195 / D14[a]	2591 (+7)	2592 (+3)	2593 (+3)	2594 (+2)	2593 (+3)	2591 (+6)
PCB 194	2639 (+6)		2642 (+3)	2642 (+3)	2643 (+3)	2641 (+7)

a. Compounds co-eluted.

Table 3.17 (contd.)

	Starting temp. 80°C		100°C	Starting temp. 120°C	
α HCH	1632 (+2)	1633 (-1)	1634 (+1)	1641 (±1)	1641 (±1)
β HCH	1660 (+2)	1661 (-1)	1663 (-1)	1668 (-2)	1671 (±1)
γ HCH	1689 (+2)	1690 (-1)	1692	1696 (±1)	1696 (±1)
Heptachlor	1846 (+2)	1846 (+1)	1847 (+1)	1852 (+2)	1852 (±1)
α Chlordene	1856 (+2)	1856	1856 (+1)	1860 (+2)	1860 (+1)
D7	1864 (+3)	1865	1865 (+1)	1867 (+2)	1867 (+2)
γ Chlordene	1904 (+2)	1904 (+1)	1905	1909 (+2)	1910 (±1)
Hept.Epox.	1982 (+3)	1982 (+2)	1983 (+1)	1987 (+2)	1987 (+2)
Oxychlordane	1990 (+2)	1990 (+1)	1991	1995 (+2)	1995 (±1)
γ chlordane	2026 (+3)	2027 (+1)	2027 (+1)	2031 (+2)	2031 (+2)
Endosulphan 1	2051 (+3)	2052 (+2)	2052 (+1)	2055 (+3)	2056 (±1)
2,4 DDE [a])					
α Chlordane)	2061 (+3)	2061 (+2)	2062	2065 (+2)	2065 (+2)
tr. Nonachlor	2078 (+3)	2078 (+2)	2080 (+1)	2083 (+3)	2083 (+2)
Dieldrin	2102 (+3)	2102 (+2)	2104	2107 (+3)	2107 (-1)
4,4 DDD	2123 (+3)	2124 (+1)	2125 (+1)	2127 (+3)	2127 (+2)
Endrin	2136 (+3)	2137 (+1)	2139	2142 (+3)	2142 (+2)
2,4 DDD	2188 (+4)	2189 (+2)	2191 (+1)	2193 (+3)	2194 (+2)
4,4 DDT	2203 (+4)	2204 (+1)	2206 (+1)	2208 (+3)	2208 (+2)
2,4 DDT	2274 (+3)	2274 (+3)	2276 (+1)	2279 (+4)	2278 (±2)
D12	2380 (+3)	2382 (+3)	2384 (+1)	2385 (+4)	2386 (+2)
PADS	2482 (+5)	2482 (+3)	2484 (+1)	2487 (+4)	2487 (+3)
cis perm	2590 (+5)	2592 (+2)	2593 (+1)	2591 (+5)	2592 (+3)
tr. perm	2622 (+5)	2624 (+2)	2625 (+1)	2624 (+5)	2625 (±3)

Solution S2.
a co-eluted

Table 3.18a Retention indices of DCBEs using a CPSil8-CB column, a starting temperature of 80°C, a temperature programme of 3 deg min^{-1} and different DCBEs as internal standards and calibration standards.

		Injection A							
		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	621.2	1383	1383	1382	1383	1382	1383	1381	1383
D3	810.6	1476	1476	1475	1476	1475	1476	1475	1476
D4	1018.5	1576	1576	1576	1576	1576	1576	1576	1576
D5	1229.8	1677	1677	1679	1677	1679	1677	1679	1677
D6	1438.5	1760	1780	1779	1780	1780	1779	1779	1779
D7	1642.9	1884	1883	1884	1883	1884	1884	1883	1883
D8	1841.8	1987	1987	1987	1987	1987	1987	1986	1986
D9	2033.2	2090	2090	2090	2090	2091	2090	2091	2090
D10	2217.8	2195	2195	2195	2195	2194	2195	2195	2195
D11	2395.0	2299	2299	2299	2299	2299	2299	2299	2299
D12	2567.7	2404	2404	2404	2404	2404	2404	2403	2403
D13	2734.2	2509	2509	2509	2509	2510	2509	2510	2509
D14	2893.6	2615	2615	2615	2615	2615	2615	2615	2614
D15	3046.6	2717	2717	2719	2717	2718	2719	2719	2719
D16	3195.1	2825	2825	2824	2824	2824	2824	2825	2825

Table 3.18a (contd.)

Ether	Time (sec)	No Int. Std. Complete Series	No Int. Std. Incomplete Series	Injection B D7 Complete Series	Injection B D7 Incomplete Series	Injection B D12 Complete Series	Injection B D12 Incomplete Series	Injection B D14 Complete Series	Injection B D14 Incomplete Series
D2	618.7	1382	1382	1380	1382	1380	1382	1380	1382
D3	808.4	1475	1475	1474	1475	1474	1475	1474	1475
D4	1016.7	1575	1575	1575	1575	1575	1575	1575	1575
D5	1228.2	1676	1676	1676	1676	1676	1676	1678	1676
D6	1437.5	1779	1779	1779	1779	1779	1779	1779	1779
D7	1642.3	1883	1883	1884	1883	1883	1883	1883	1883
D8	1841.3	1986	1989	1987	1987	1987	1987	1987	1987
D9	2032.6	2090	2090	2091	2090	2090	2090	2091	2090
D10	2216.6	2194	2194	2194	2194	2194	2194	2194	2194
D11	2394.8	2298	2299	2299	2299	2298	2299	2299	2299
D12	2567·0	2403	2404	2404	2404	2404	2404	2404	2404
D13	2732.6	2508	2509	2509	2509	2509	2508	2509	2509
D14	2892.6	2614	2615	2615	2615	2614	2614	2615	2614
D15	3046.1	2717	2718	2719	2718	2719	2719	2719	2719
D16	3795.3	2825	2826	2825	2826	2825	2825	2825	2825

Table 3.18b Retention indices of DCBEs using a CPSil8-CB column, a starting temperature of 100°C, a temperature rate of 3 deg min^{-1} and different DCBEs as internal standards and calibration standards.

Injection A

		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	389.8	1388	1386	1388	1386	1387	1386	1388	1386
D3	528.2	1479	1488	1479	1488	1479	1488	1479	1488
D4	699.5	1579	1579	1579	1579	1579	1579	1579	1579
D5	888.6	1681	1678	1680	1678	1680	1678	1681	1678
D6	1084.8	1782	1782	1782	1782	1782	1782	1782	1782
D7	1283-0	1885	1885	1884	1884	1885	1885	1884	1885
D8	1477.5	1987	1986	1987	1987	1987	1987	1987	1987
D9	1667.5	2091	2090	2091	2090	2090	2090	2091	2090
D10	1851.6	2195	2195	2195	2194	2194	2195	2195	2195
D11	2030-0	2299	2299	2299	2299	2298	2299	2299	2299
D12	2202.4	2405	2405	2405	2405	2405	2405	2405	2405
D13	2369.1	2510	2510	2510	2510	2510	2510	2510	2510
D14	2528.6	2615	2615	2615	2615	2615	2615	2615	2615
D15	2682.6	2719	2718	2719	2719	2719	2719	2719	2719
D16	2832.7	2825	2825	2825	2825	2825	2825	2825	2825

Table 3.18b (contd.)

Injection B

		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	390.4	1388	1386	1388	1388	1388	1386	1387	1386
D3	528.1	1478	1488	1478	1488	1478	1488	1478	1487
D4	699.5	1579	1579	1578	1578	1578	1578	1578	1578
D5	868.7	1681	1679	1680	1678	1680	1678	1680	1678
D6	1085.6	1782	1782	1781	1781	1781	1781	1781	1781
D7	1284.1	1885	1885	1884	1884	1884	1884	1884	1884
D8	1479.3	1987	1987	1987	1987	1986	1986	1986	1986
D9	1669.6	2092	2091	2091	2090	2090	2090	2090	2090
D10	1854.3	2196	2196	2195	2194	2194	2194	2194	2194
D11	2033.0	2300	2301	2299	2299	2299	2299	2299	2299
D12	2205.7	2407	2407	2405	2405	2404	2404	2404	2404
D13	2372.6	2512	2512	2510	2510	2509	2510	2509	2510
D14	2532.7	2617	2617	2616	2615	2615	2615	2615	2615
D15	2686.7	2721	2721	2720	2719	2719	2719	2718	2719
D16	2836.5	2824	2826	2825	2825	2824	2824	2824	2824

Table 3.18c Retention indices of DCBEs using a CPSil8-CB column, a starting temperature of 120°C, a temperature programme of 3 deg min^{-1} and different DCBEs as internal standards and calibration standards.

Injection A

		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	237.9	1400	1453	1400	1453	1400	1453	1400	1453
D3	320-0	1485	1507	1485	1507	1485	1507	1485	1507
D4	436.3	1584	1585	1585	1585	1585	1585	1584	1584
D5	580.3	1685	1681	1685	1681	1681	1681	1685	1681
D6	744.9	1784	1785	1785	1785	1785	1785	1785	1785
D7	922.8	1886	1886	1886	1887	1887	1887	1887	1887
D8	1105.4	1989	1989	1989	1989	1989	1989	1988	1988
D9	1287.7	2092	2092	2092	2091	2092	2091	2091	2091
D10	1488.2	2196	2196	2196	2196	2196	2196	2196	2196
D11	1644.6	2300	2300	2299	2300	2300	2300	2299	2300
D12	1815.9	2405	2405	2405	2405	2404	2405	2405	2404
D13	1982.2	2510	2510	2510	2510	2510	2510	2510	2510
D14	2141.7	2615	2615	2615	2615	2615	2615	2615	2615
D15	2296-0	2719	2719	2719	2716	2719	2719	2719	2719
D16	2445.8	2825	2825	2825	2825	2825	2825	2825	2825

Table 3.18c (contd.)

Injection B

Ether	Time (sec)	No Int. Std. Complete Series	No Int. Std. Incomplete Series	D7 Complete Series	D7 Incomplete Series	D12 Complete Series	D12 Incomplete Series	D14 Complete Series	D14 Incomplete Series
D2	238.6	1400	1453	1400	1453	1400	1453	1400	1453
D3	320.5	1485	1507	1485	1507	1485	1507	1485	1507
D4	436.9	1585	1585	1585	1585	1585	1585	1585	1585
D5	581.3	1685	1681	1685	1681	1685	1681	1685	1681
D6	745.2	1785	1785	1785	1785	1785	1785	1785	1785
D7	922.9	1887	1887	1887	1887	1886	1886	1886	1886
D8	1105.2	1988	1988	1988	1988	1988	1988	1988	1988
D9	1287.7	2091	2091	2091	2091	2091	2091	2091	2091
D10	1468.2	2196	2196	2195	2195	2195	2195	2195	2195
D11	1646.6	2300	2300	2299	2300	2299	2300	2300	2300
D12	1816.1	2405	2405	2405	2405	2404	2405	2404	2404
D13	1982.4	2510	2510	2509	2510	2509	2510	2509	2510
D14	2142·0	2615	2615	2615	2615	2615	2615	2615	2615
D15	2296.7	2719	2720	2719	2717	2719	2719	2719	2720
D16	2446.3	2825	2825	2825	2825	2825	2825	2825	2825

Table 3.19 Retention indices of organochlorine compounds using a CPSil8-CB column, a temperature programme of 3 deg min^{-1} and different starting temperatures.

	Starting temp. 80°C				Starting temp. 100°C				Starting temp. 120°C			
HCB	1681	(-2)	1681	(-3)	1687	(±2)	1696	(-4)	1696	(-4)		
PCB28	1849	(-1)	1850	(-1)	1854	(-2)	1857	(-1)	1857	(-1)		
Heptachlor	1868		1868	(-1)								
D7	1884	(-1)	1884	(-1)	1884	(±2)	1887	(-1)	1887	(-1)		
PCB52	1920		1920		1921	(+2)	1925	(-1)	1925	(-1)		
Aldrin	1930		1930	(-1)	1932	(+2)	1938	(-1)	1938	(-1)		
PCB44	1958		1958	(-1)	1959	(+2)	1963	(-1)	1963	(-1)		
2,4 DDE	2088		2089	(-2)	2087	(+2)	2091	(-1)	2091	(-1)		
PCB 101	2093	(-1)	2093	(-1)	2093	(+2)	2096	(-1)	2096	(-1)		
4,4 DDE	2157	(-1)	2157	(-1)	2157	(+2)	2159	(-1)	2159	(-1)		
PCB118	2219		2219	(-1)	2220	(+2)	2222	(-1)	2222	(-1)		
PCB153	2268		2269	(-1)	2269	(+3)	2271	(-2)	2271	(-1)		
PCB137	2307		2307	(-1)	2307	(+3)	2309	(±1)	2309	(±1)		
PCB138	2324		2325	(-1)	2326	(+2)	2327	(-2)	2327	(-1)		
PCB128	2380		2380	(-1)	2382	(+2)	2383	(-1)	2383	(-1)		
PCB180	2473		2474	(-2)	2474	(+3)	2476	(-2)	2476	(-2)		
Mirex	2495	(-1)	2495	(-2)	2509	(+3)	2499	(-2)	2498	(-2)		
D14	2614		2615	(-2)	2614	(+3)	2616	(-2)	2616	(-2)		
PCB195	2633	(-1)	2633	(-2)	2634	(+3)	2636	(-3)	2635	(-1)		
PCB194	2682	(-1)	2683	(-3)	2683		2685	(-3)	2684	(-1)		

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Table 3.19 (contd.)

	Starting temp. 80°C		Starting temp. 100°C		Starting temp. 120°C			
α HCH	1674	(-1)	1679	(+3)	1687	(-4)	1686	(-3)
β HCH	1731	(-1)	1734	(+2)	1740	(-2)	1739	(-1)
γ HCH	1737	(-1)	1740	(±1)	1747	(-2)	1746	(-1)
Heptachlor	[a] 1868							
D7	1884	(-1)	1885	(-2)	1887	(-1)	1887	(-1)
α Chlordene					1893	(-1)	1892	
γ Chlordene	1942		1944	(+1)	1948		1948	
Hept. Epox	[a] 2016	(-1)	2017	(+2)	2021	(-1)	2021	
Oxychlordane								
γ Chlordane	2062		2063	(+2)	2067	(-1)	2066	
Endosulphan 1	2083	(-1)	2083	(±2)	2087	(-1)	2086	
2,4 DDE	2089		2089	(+2)	2091		2091	
α Chlordane	2094		2094	(+3)	2097	(+1)	2097	
Tr Nonachlor	2104		2105	(+3)	2108		2108	
Dieldrin	2136		2137	(+2)	2140	(-1)	2140	(-1)
2,4 DDD	[a] 2171		2171	(+2)	2173	(-1)	2173	(-1)
Endrin	2177							
4,4 DDD	[a] 2243		2243	(+3)	2245		2245	(+1)
2,4 DDT								
4,4 DDT	2320		2319	(+3)	2321	(-1)	2320	
D12	2405		2405	(+3)	2405		2405	(-1)
PADS	2538		2537	(+3)	2538		2538	
cis perm	2682	(-1)	2679	(+4)	2680	(-1)	2680	(-1)
tr perm	2700	(-1)	2699	(-4)	2700	(-1)	2699	(-1)

Solution S2

a co-eluted

Table 3.20a Retention indices of DCBEs using a CPSil8-CB column, a starting temperature of 80°C, a temperature programme of 5 deg min^{-1} and different DCBEs as internal standards and calibration standards.

		No Int. Std.		D7		D12		D14	
Ether	Time (sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	521.1	1400	1391	1400	1391	1399	1391	1400	1391
D3	643.9	1487	1485	1486	1485	1487	1485	1487	1485
D4	778.6	1587	1587	1586	1587	1587	1587	1587	1587
D5	912.7	1688	1688	1688	1688	1688	1688	1688	1688
D6	1044.1	1789	1789	1789	1789	1789	1789	1789	1789
D7	1171.6	1891	1891	1890	1890	1891	1891	1891	1891
D8	1292.9	1994	1994	1994	1994	1994	1993	1994	1994
D9	1411.8	2097	2097	2097	2097	2097	2097	2097	2097
D10	1525.6	2200	2200	2200	2200	2200	2200	2200	2200
D11	1635.2	2305	2305	2305	2305	2305	2305	2305	2305
D12	1740.4	2411	2411	2411	2411	2411	2411	2411	2411
D13	1841.8	2516	2516	2515	2516	2516	2516	2516	2516
D14	1939.6	2622	2621	2621	2621	2621	2621	2621	2622
D15	2031.9	2725	2727	2725	2725	2726	2726	2726	2727
D16	2125.2	2832	2831	2831	2831	2832	2832	2832	2832

Table 3.20b Retention indices of DCBEs using a CPSil8-CB column, a starting temperature of 100°C, a temperature programme of 5 deg min^{-1} and different DCBEs as internal standards and calibration standards.

		No Int. Std.		D7		D12		D14	
	Time (Sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	339·0	1400	1411	1399	1411	1400	1411	1399	1411
D3	436.4	1487	1491	1487	1491	1487	1491	1487	1491
D4	552.6	1587	1587	1587	1587	1587	1587	1586	1587
D5	675.1	1688	1687	1688	1687	1688	1687	1688	1687
D6	798.9	1789	1789	1788	1788	1789	1789	1789	1788
D7	922·0	1891	1891	1891	1891	1891	1890	1891	1891
D8	1040.3	1994	1994	1994	1994	1993	1993	1994	1994
D9	1157.7	2097	2097	2097	2097	2097	2097	2097	2097
D10	1269.9	2200	2200	2199	2200	2200	2200	2200	2200
D11	1378.7	2305	2305	2305	2305	2305	2305	2305	2305
D12	1483.4	2411	2411	2410	2410	2410	2411	2410	2411
D13	1583.5	2516	2516	2515	2516	2516	2516	2515	2516
D14	1690.1	2622	2622	2622	2622	2622	2622	2621	2621
D15	1774·0	2725	2728	2726	2724	2725	2725	2725	2727
D11	1864.7	2832	2832	2832	2832	2831	2831	2832	2832

Table 3.20c Retention indices of DCBEs using a CPSil8-CB column, a starting temperature of 120°C, a temperature programme of 5 deg min^{-1} and different DCBEs as internal standards and calibration standards.

		No Int. Std.		D7		D12		D14	
	Time (Sec)	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series	Complete Series	Incomplete Series
D2	225.0	1400	1437	1400	1437	1400	1437	1400	1437
D3	292.6	1487	1488	1487	1488	1486	1488	1486	1488
D4	381.6	1586	1587	1587	1587	1586	1587	1586	1586
D5	484.4	1687	1685	1688	1685	1688	1685	1687	1685
D6	595.6	1789	1789	1789	1789	1789	1789	1789	1789
D7	710.7	1891	1891	1891	1891	1891	1891	1891	1891
D8	825.6	1994	1994	1994	1994	1994	1994	1993	1994
D9	940.1	2097	2097	2097	2097	2097	2097	2097	2097
D10	1051.6	2200	2200	2200	2200	2200	2199	2200	2200
D11	1160.6	2305	2305	2305	2305	2305	2305	2305	2305
D12	1265.0	2411	2411	2411	2411	2410	2410	2411	2411
D13	1365.8	2516	2516	2516	2516	2516	2516	2516	2516
D14	1463.7	2621	2622	2622	2622	2622	2622	2622	2622
D15	1557.9	2726	2726	2726	2726	2726	2726	2726	2727
D16	1649.3	2832	2832	2832	2832	2832	2832	2832	2832

Table 3.21 Retention indices of organochlorine compounds using a CPSil8-CB column, a temperature programme of 5 deg min^{-1} and different starting temperatures.

	Starting temp. 80°C				Starting temp. 100°C				Starting temp. 120°C			
HCB	1697		1697	(-1)	1699	(+1)	1699	(+2)	1703	(-1)	1702	(-3)
PCB 28	1861		1862	(-1)	1866	(-3)	1866	(-2)	1864	(-1)	1864	(-2)
Heptachlor	1882	(-1)	1882	(-1)	1883	(+1)	1885	(-1)				
D7	1890	(+1)	1890		1891	(+1)	1891	(+2)	1891	(-1)	1890	(±1)
PCB 52	1931		1931	(-1)	1931	(+)	1931	(+2)	1932		1932	(-1)
Aldrin	1947	(+1)	1946		1948	(+1)	1948	(+2)	1950	(-1)	1950	(-1)
PCB 44	1970		1970		1971	(+1)	1971	(+2)	1972	(-1)	1972	(-1)
2,4 DDE	2100		2100	(-1)	2100	(+2)	2099	(+3)	2101	(-1)	2101	(-1)
PCB 101	2105	(-1)	2104		2104	(+2)	2104	(+3)	2105	(+1)	2106	(-2)
4,4 DDE	2167	(-1)	2166		2166	(+2)	2166	(+1)	2167	(-1)	2168	(-2)
PCB 118	2231		2231	(-1)	2231	(+1)	2231	(+3)	2232	(-1)	2233	(-2)
PCB 153	2281		2281	(-1)	2280	(+2)	2280	(+2)	2282	(-1)	2283	(-3)
PCB 137	2322	(-1)	2321	(-1)	2320	(+2)	2320	(+2)	2322	(-1)	2323	(-2)
PCB 138	2339	(+1)	2339	(-1)	2338	(+2)	2338	(+1)	2340	(-1)	2341	(-2)
PCB 128	2398	(-1)	2397	(-1)	2396	(+2)	2395	(+2)	2398	(+1)	2399	(-2)
PCB 180	2490	(-1)	2490	(-2)	2488	(+2)	2488	(+2)	2490	(-1)	2491	(-2)
Mirex	2523		2523	(±1)	2523	(±2)	2522	(+1)	2524	(-1)	2525	(-3)
D14	2622		2622	(-1)	2620	(+3)	2620	(+1)	2622	(-1)	2623	(-3)
PCB 195	2653		2654	(-2)	2652	(+2)	2651	(+2)	2654	(-2)	2655	(-3)
PCB 194	2702		2702	(-2)	2699	(+3)	2699	(+3)	2702	(-2)	2703	(-3)

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Table 3.21 (contd.)

	Starting temp. 80°C		Starting temp. 100°C		Starting temp. 120°C		
α HCH	1688		1690	(+2)	1692	(+2)	1692
β HCH	1743		1744	(+2)	1745	(+1)	1745
γ HCH	1750		1751	(+2)	1753		1754
Heptachlor	1882		1883	(+3)	1883		1883
D7	1891		1890		1890		1890
γ Chlordene	1899	(+1)	1902	(-2)	1901		1901
α Chlordene	1956	(-1)	1960	(-2)	1958		1958
Hept. Epox [a]	2032	(-1)	2033	(+1)	2034		2034
Oxychlordane [a]							
γ Chlordone	2078	(-1)	2078	(+1)	2080		2080
Endosulphanl	2100	(-1)	2100	(+1)	2101	(-1)	2101
2,4 DDE [a]							
α Chlordane [a]	2109	(-1)	2109	(+1)	2111		2111
Tr Nonochlor	2120	(-1)	2120	(±1)	2121		2121
Dieldrin	2152		2152	(+1)	2154		2154
2,4 DDD [a]	2182	(-1)	2181		2182		2182
Endrin [a]		(-1)		(+1)			
4,4 DDD [a]	2253		2252		2254		2254
2,4 DDT [a]		(-1)		(+1)			
4,4 DDT	2332	(-1)	2331		2333		2332 (-1
D12	2412	(-2)	2409	(+1)	2411	(-1)	2410
PADS	2254	(-2)	2551	(+1)	2553		2553
cis perm	2690	(-1)	2686	(+2)	2689	(-1)	2689 (-1
tr perm	2709	(-2)	2705	(+1)	2708	(+1)	2708 (-1

Solution S2.

a- co-eluted

3.6 GEL PERMEATION CHROMATOGRAPHY

3.6.1 Examination of Test Solutions of Highly Refined Cod Liver Oil

The lipid and organochlorine profiles were examined at different flow rates using the solution described in Section 2.10.2. The results are summarised in Table 3.22 shown below.

Table 3.22 Time and volume of solvent required for elution of lipid and organochlorine residues from highly refined cod liver oil + Hexachlorobenzene (HCB) and Decachlorobiphenyl (DCBP).

Flow Rate ml/min^{-1}	Elution Conditions Lipid Fraction		Elution Conditions Organochlorine Fraction	
	Time (min)	Vol (ml)	Time (min)	Vol (ml)
1	43.0	43.0	25.0	25
2	22.8	45.6	14.6	29.2
3	15.4	46.2	12.6	25.2
4	11.55	46.2	7.4	29.6
5	9.6	48.0	5.8	29.0

3.6.2 Measurement of Non-volatile Residues in Highly Refined Cod Liver Oil Solution

The non-volatile residues present were measured (using the solution described in Section 2.10.3) and the results are summarised in Table 3.23 shown below. The bulk of the lipid fraction corresponded to the fraction eluting between 30-55ml whereas the smaller molecular weight residues corresponded to the fraction eluting after 55ml.

Table 3.23 Mass of non-volatile lipid residues in highly refined cod liver oil.

Injection	Sample Concentration mg ml^{-1}	Fraction collected (ml)	Mass of Lipid (mg)
1	100	34-90	109.5
2	100	34-90	101.4
3	100	34-90	101.9

		Fraction 1	Fraction 2*	Fraction 1	Fraction 2
1	100	30-55	55-90	101.1	4.3
2	100	30-55	55-90	104.4	3.9

* Two separate fractions collected in the ranges stated.

3.6.3 Measurement of Organochlorine Residues in Highly Refined Cod Liver Oil

The mass of non-volatile lipid residue present in the test injections is given below. Details of the gas chromatographic analysis are given in Section 3.7.1.

Mass of non-volatile lipid residue in fraction 86-136 ml.

injection 1 0.4 mg

injection 2 0.4 mg

3.6.4 Test Injections and Measurement of Non-volatile Residues in Refined Capelin Oil

The volume of solvent and analysis time required for the elution of the lipid and organochlorine fractions at different flow rates is shown in Table 3.24. The mass of non-volatile residues present in the lipid and organochlorine fractions is shown in Table 3.25.

Table 3.24 Time and volume of solvent required for the elution of lipid and organochlorine fractions.

Sample	Flow Rate ml min^{-1}	Volume solvent eluted before lipid (ml)	Volume solvent for elution lipid (ml) Peak 1	Peak 2	Elution volume (ml) organochlorine
Refined capelin oil	3	37.8	33.0	48.0	
Refined capelin oil + HCB + DCBP	1	26.6	33.2	21.4	25
Ditto	2	35.2	31.6	22	26
Ditto	3	37.2	31.8	21.6	27

Table 3.25 Table showing mass of non-volatile residue in lipid and organochlorine fraction in refined capelin oil.

Sample	Fraction collection (ml)		Residue Mass (mg) Fraction 1	Fraction 2
Refined capelin oil	37-70	70-170	98.8	1.1
Ditto	37-70	70-170	100.7	1.1
Ditto	37-70	70-170	102.0	1.4
Refined capelin oil	37-84	84-184	101.4	0.3
Ditto	37-84	84-184	103.7	0.5
Ditto	37-84	84-184	105.9	0.2

Sample loading 100mg.

3.6.5 Examination of 400 mgl^{-1} Fish Oil Solutions

The volume of solvent required for the elution of the lipid fraction in the fish oil solutions described in Section 2.10.10 of Chapter 2 is shown in Table 3.26. The volume of solvent required for the elution of hexachlorobenzene and decachlorobiphenyl under similar conditions is also shown.

3.6.6 Measurement of Non-volatile Residues in Fish Oils

The mass of non-volatile residue present in the eluate corresponding to the organochlorine fraction was measured for each of the four sample oils. The results are summarised in Table 3.27. The sample loading was 400mg.

Table 3.26 Solvent volumes required for elution of lipid and organochlorine fractions in fish oils.[a]

Sample	Loading	Total volume solvent for elution (ml)	Lipid volume (ml)	Total time for elution (min)
Highly refined cod liver oil	400 mg	96	63	32
Refined capelin oil	400 mg	105	70.8	35
Crude capelin oil	400 mg	120	84	40
Crude cod liver oil	400 mg	108	78	36
Decachlorobiphenyl	40 µg	102		34
Hexachlorobenzene	40 µg	108		36

a Injection volume 2ml.

Table 3.27 Table showing the mass of non-volatile residue in eluate corresponding to the organochlorine fraction. [a]

Sample	Residue Mass mg
Crude Cod Liver Oil	3.3
Ditto	5.0
Ditto	4.6
Crude Capelin Oil	4.4
Ditto	5.5
Ditto	4.3
Refined Capelin Oil	4.2
Ditto	4.7
Ditto	3.6
Highly Refined Cod Liver Oil	3.9
Ditto	1.2
Ditto	1.4

a fraction collected 90-140ml

3.7. GAS CHROMATOGRAPHIC ANALYSIS OF FISH OIL SAMPLES

3.7.1 Highly Refined Cod Liver Oil

Examination of the samples described in Section 2.10.4 of Chapter 2 using gas chromatography was unsuccessful; the concentration of any organochlorines present was too low to allow accurate quantitation. No quantifiable ($<0.2\mu g\ kg^{-1}$) residues were present in any of the blank injections.

3.7.2 Residues of Organochlorine Compounds Present in Fish Oil Samples

The samples described in Section 2.10.11 were examined by gas chromatography using either a CPSil5-CB or a CPSil8-CB column. Retention indices and peak identities were assigned as described using an 'identification window' of 3 index units (Section 2.9.3).

The quantities of organochlorines present in the eluate 1A for each of the fish oils are shown in Tables 3.28a-3.28e using a CPSil5-CB column and Table 3.29 for the results obtained using a CPSil8-CB column. The quantities present in the eluate 1B+2 are shown in Tables 3.30a-3.30c for the fish oils and blank injections using a CPSil5-CB column and Table 3.31 for a CPSil8-CB column.

In those cases where peaks were unresolved in the standard solutions both identities are given. The quantities were calculated using both internal standards, the external standard concentration was $0.1mg\ l^{-1}$ for the CPSil5-CB column and $0.02mg\ l^{-1}$ and $0.1mg\ l^{-1}$ for the CPSil8-CB column. No quantifiable residues were found in Eluate 1B+2 of the Highly Refined Cod Liver Oil or the 'Blank' injections. Where a compound was not identified in a sample no value is given in the Table.

Table 3.28a Quantities in $\mu g\ kg^{-1}$ of organochlorine residues present in eluate 1A of highly refined cod liver oil using a CPSil5-CB column.

Sample Compound chosen as Internal Standard	Sample I		Sample II		Sample III	
	D7	D14	D7	D14	D7	D14
HCB	0.24	0.24	0.21	0.19	0.22	0.20
D7	12.50	12.14	12.50	11.22	12.50	11.66
PCB52	0.20	0.20				
PCB44	0.75	0.73	1.50	1.35	0.74	0.74
4,4DDE	0.20	0.20	0.17	0.15		
PCB118	0.22	0.22				
PCB153	2.03	1.98	3.52	3.16	2.30	2.14
PCB138	0.27	0.26	0.23	0.21	0.21	0.20
PCB180	0.29	0.28	0.30	0.27	0.29	0.27
D14	12.87	12.50	13.92	12.5	13.40	12.50

Table 3.28b Organochlorine residues present in refined capelin oil.

Sample Compound chosen as Internal Standard	Sample I		Sample II		Sample III	
	D7	D14	D7	D14	D7	D14
HCB	1.14	0.99	1.05	1.11	1.20	1.22
D7	12.50	10.82	12.50	13.13	12.50	12.71
PCB52	0.45	0.39			0.44	0.45
PCB44	1.39	1.20	1.33	1.39	1.32	1.34
4,4DDE	0.70	0.61	0.53	0.56	0.81	0.83
PCB118	0.43	0.38	0.31	0.33	0.46	0.47
PCB153	3.09	2.68	2.73	2.87	2.62	2.67
PCB138	0.34	0.29	0.25	0.26	0.34	0.34
D14	14.14	12.50	11.90	12.50	12.29	12.50

Table 3.28c Organochlorine residues present in eluate 1A of crude capelin oil using a CPSil5-CB column.

Sample Compound chosen as Internal Standard	Sample I		II		III	
	D7	D14	D7	D14	D7	D14
HCB	2.15	2.05	2.49	2.03	2.22	1.92
PCB28	0.43	0.41	0.51	0.41	0.47	0.41
D7	12.50	11.88	12.50	10.18	12.50	10.80
PCB52	0.95	0.90	1.00	0.83	0.96	0.83
PCB42	1.29	1.23	1.26	1.03	1.13	0.98
PCB101	1.04	0.99	1.21	1.16	1.00	1.06
4,4DDE	3.59	3.42	4.02	4.11	3.56	3.56
PCB118	1.37	1.30	1.66	1.59	1.38	1.36
PCB153	3.21	3.05	2.71	2.37	2.05	1.94
PCB138	1.19	1.13	1.37	1.39	1.20	1.00
PCB128	0.60	0.58	0.67	0.64	0.56	0.51
PCB180	0.54	0.51	0.69	0.64	0.54	0.50
D14	13.15	12.50	15.35	12.50	13.40	12.50

Table 3.28d Organochlorine residues present in eluate 1A of crude cod liver oil using a CPSil5-CB column.

Sample Compound chosen as Internal Standard	Sample I		Sample II		Sample III	
	D7	D14	D7	D14	D7	D14
HCB	3.55	3.32	2.93	2.76	2.79	2.21
PCB28	0.63	0.59	0.54	0.51	0.56	0.45
D7	12.50	11.63	12.50	11.79	12.50	9.89
PCB52	1.21	1.33	1.16	1.10	1.21	0.96
PCB44	0.54	0.51	0.93	0.88	0.87	0.69
PCB101	1.62	1.52	1.41	1.33	1.52	1.20
4,4DDE	3.78	3.53	4.62	4.36	4.91	3.91
PCB118	2.42	2.27	2.24	2.11	2.20	1.74
PCB153	2.91	2.72	3.08	2.91	3.10	2.34
PCB138	2.25	2.10	2.15	2.03	2.02	1.70
PCB128	0.87	0.82	0.77	0.72	0.76	0.60
PCB180	1.05	0.98	1.02	0.96	1.02	0.81
D14	13.34	12.50	13.26	12.50	15.80	12.50

Table 3.28e Organochlorine residues present in eluate 1A of 'Blank' injections using a CPS115-CB column.

Sample Compound Chosen as Internal Standard	Sample I		Sample II	
	D7	D14	D7	D14
D7	12.50	13.72	12.50	13.15
PCB44	0.97	1.06	1.23	1.30
PCB153	1.76	1.93	2.26	2.72
D14	11.39	12.50	11.88	12.50

Table 3.29 Organochlorine residues in eluate 1A of fish oils using a CPSil8-CB column.

Sample compound Chosen as Internal Standard	Highly Refined Cod Liver Oil		Refined Capelin Oil		Crude Capelin Oil		Crude Cod Liver Oil		Blank A		Blank B	
	D7	D14	D7	D14	D7	D14	D7	D14	D7	D14	D7	D14
HCB			0.64	0.70	1.14	1.44	1.83	2.16				
D7	12.50	14.71	12.50	13.61	12.50	15.85	12.50	14.76	12.50	14.09	12.50	17.60
PCB52							0.36	0.94				
PCB44							0.20	0.24				
2,4DDE							0.41	0.48				
PCB101			0.20	0.22	0.74	0.94	1.24	1.47				
4,4DDE			0.66	0.72	3.39	4.30	2.70	3.19				
PCB118			0.25	0.28	0.88	1.11	1.51	1.78				
PCB153			0.38	0.42	1.38	1.68	2.59	2.06				
PCB138			0.33	0.36	0.95	1.20	1.90	2.25				
PCB180					0.47	0.60	0.87	1.03				
D14	10.62	12.50	11.48	12.50	9.86	12.50	10.58	12.50	11.08	12.50	9.2	12.50

Table 3.30a Organochlorine residues present in eluate 1B+2 refined capelin oil using a CPSil5-CB column.

Sample Compound chosen as Internal Standard	I		II		III	
	D7	D12	D7	D12	D7	D12
α HCH	0.80	0.79	0.78	0.73	0.81	0.80
D7	12.50	12.36	12.50	11.08	12.50	13.32
Hept. Epox	0.31	0.30	0.25	0.24	0.30	0.32
Y-Chlordane	0.29	0.28	0.30	0.29	0.32	0.34
2,4-DDE	0.94	0.93	0.85	0.80	0.97	1.03
α-Chlordane	0.65	0.64	0.59	0.56	0.67	0.71
tr-Nonachlor	0.64	0.63	0.57	0.54	0.59	0.63
Dieldrin	0.23	0.22			0.27	0.29
2,4-DDD	0.56	0.55	0.49	0.47	0.51	0.54
4,4-DDD	1.04	1.03	0.90	0.85	0.99	1.04
2,4-DDT	0.50	0.49	0.31	0.30	0.35	0.38
4,4-DDT	0.88	0.85	0.68	0.64	0.80	0.85
D12	12.66	12.50	13.24	12.50	11.73	12.50

Table 3.30b Organochlorine residues in eluate 1B+2 crude cod liver oil using a CPSil5-CB column.

Sample Compound Chosen as Internal Standard	I		II	
	D7	D12	D7	D12
α-HCH	0.60	0.59	0.55	0.59
α-chlordene	0.28	0.28	0.20	0.22
D7	12.50	12.53	12.50	13.57
Hept Epox	0.55	0.50	0.44	0.47
Oxychlordane	0.60	0.59	0.52	0.57
γ-Chlordane	0.83	0.81	0.70	0.76
Endos 1	0.25	0.24	0.38	0.41
2,4-DDE	3.35	3.31	3.10	3.33
α-Chlordane	2.32	2.29	2.12	2.30
tr-Nonachlor	2.78	2.74	2.50	2.71
Dieldrin	0.35	0.32	0.25	0.27
2,4-DDD	1.38	1.36	1.36	1.46
Endrin	0.61	0.60	0.47	0.53
4,4 DDD	6.66	6.57	6.37	6.92
2,4 DDT	1.12	1.11	0.97	1.05
4,4 DDT	2.14	2.11	1.85	2.00
D12	12.67	12.50	11.51	12.50

Table 3.30c Organochlorine residues in eluate 1B+2 of crude capelin oil on a CPSil5-CB column.

Sample Compound chosen as Internal Standard	I		II		III	
	D7	D12	D7	D12	D7	D12
α-HCH	0.51	0.53	0.53	0.51	0.51	0.50
D7	12.50	13.18	12.50	11.91	12.50	12.24
Hept Epox	0.40	0.47	0.46	0.44	0.48	0.47
oxychlordane	0.22	0.24	0.26	0.25	0.27	0.27
γ-Chlordane	0.59	0.62	0.62	0.61	0.65	0.63
2,4-DDE	1.82	1.92	1.96	1.88	1.98	1.94
α-Chlordane	1.26	1.33	1.36	1.30	1.37	1.34
tr-Nonachlor	1.16	1.22	1.26	1.20	1.30	1.28
Dieldrin	0.26	0.22	0.21	0.21	0.72	0.71
2,4-DDD	0.87	0.92	0.96	0.92	0.96	0.96
Endrin	0.58	0.61	0.55	0.53	0.67	0.66
1,4-DDD	2.28	2.40	2.49	2.38	2.77	2.72
2,4-DDT	2.01	2.11	2.10	2.00	2.42	2.37
4,4-DDT	4.54	4.78	4.78	4.57	3.97	4.87
D12	11.86	12.50	13.08	12.50	12.76	12.50

Table 3.31 Organochlorine residues in eluate 1B+2 of fish oils using a CPS118-CB column.

Sample compound Chosen as Internal Standard	Highly Refined Cod Liver Oil		Refined Capelin Oil		Crude Capelin Oil		Crude Cod Liver Oil		Blank A		Blank B	
	D7	D12	D7	D12	D7	D12	D7	D12	D7	D12	D7	D12
α-HCH			1.17	1.37								
D7	12.50	15.11	12.50	14.66	12.50	14.07	12.50	13.75	12.50	13.06	12.50	14.10
Hept Epox			0.21	0.25	0.29	0.33	0.71	0.79				
Oxychlordane					0.29	0.33	0.70	0.78				
α-Chlordane			0.42	0.50	0.69	1.01	1.16	1.27				
Endos 1			0.65	0.80	0.86	0.96	1.18	1.30				
2,4 DDE			0.88	1.04	.170	1.92	3.12	3.43				
α-Chlordane			0.88	1.04	1.70	1.92	3.12	3.43				
tr-Nonachlor			0.73	0.85	1.38	1.56	3.39	3.73				
Dieldrin			0.27	0.32	0.33	0.37	0.20	0.20				
2,4 DDD			0.42	0.49	0.62	0.70	1.00	2.7				
Endrin			0.42	0.49	0.63	0.71	1.10	2.72				
4,4 DDD					1.41	1.59	3.08	3.38				
2,4 DDT			0.43	0.51	0.97	1.09	0.60	0.66				
4,4 DDT			0.52	0.61	3.06	3.44	1.56	1.71				
D12	10.38	12.50	10.66	12.50	11.10	12.50	11.36	12.50	11.97	12.50	10.98	12.50

CHAPTER FOUR

DISCUSSION

4.1 DEVELOPMENT AND USE OF 2,4-DICHLOROBENZYL ALKYL ETHERS

In Chapter 1 the need for a series of compounds suitable for use as both internal standards and retention index markers was discussed. To do this satisfactorily the compounds chosen must fulfil the criteria listed in 1.6, page 45, Chapter 1. The compounds chosen were a homologous series of 2,4-dichlorobenzyl alkyl ethers and the following discussion will indicate how they fulfilled the criteria set and any problems encountered in their preparation and use. There will be some discussion of the actual numerical values obtained for the retention indices of both the DCBEs and the organochlorine compounds but no detailed discussion will be attempted. To examine these compounds and their chromatographic behaviour in detail was outside the scope of the work and insufficient time was available. The objective of the work was to prepare the series of compounds and to test them using practical problems of environmental analysis - in this case an examination of residues of organochlorine compounds in fish oils both qualitatively and quantitatively.

All the gas chromatography involving the DCBEs was done over two stays of six months each in the Department of Agriculture and Fisheries for Scotland, Freshwater Fisheries Laboratory, at Pitlochry and in accordance with the protocols of this laboratory, viz the instrumentation, the sample preparation and data analysis. Thus the method of calculation for the retention indices and the conditions for gas chromatographic analysis were restricted to those used routinely in this laboratory.

The performance of the DCBEs as internal standards and retention index markers will thus be examined in terms of the criteria set down in Chapter 1.

4.2 PREPARATION OF 2,4-DICHLOROBENZYL ALKYL ETHERS

4.2.1 General Methods of Ether Preparation

There are numerous methods available for the preparation of ethers. The majority of methods rely on the conversion of an alcohol into an effective nucleophile which then displaces a leaving group from some other substrate. The most commonly used method is the Williamson ether synthesis,[155] in which the alcohol is converted into an alkoxide by means of a strong base. The alkoxide subsequently reacts with a substrate such as a haloalkane. The reaction scheme is shown below in Figure 4.1.

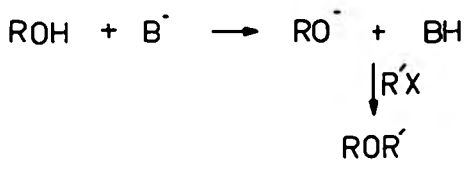


FIGURE 4.1 Reaction scheme for Williamson ether synthesis

The bromoalkanes are usually chosen since these are generally more reactive than the corresponding chloroalkanes and are cheaper and less susceptible to dehydrohalogenation than the iodoalkanes.

Alternative methods for the preparation were reviewed by Feuer and Hoos[156] and these include alkylations with dialkyl sulphates, reaction of phenolates with diphenyliodonium salts, the dehydration of alcohols in the presence of acid, the reaction of carbodi-imides with alcohols and the reduction of acetals and hemi-acetals. Onaki et al[157] suggested using zeolites which allow ether preparation under neutral conditions in non-polar solvents, the heterogenous reaction being promoted by the

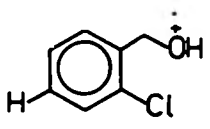
acidic and basic surface sites on the zeolite.

4.2.2 Reduction of 2,4-Dichlorobenzoic Acid

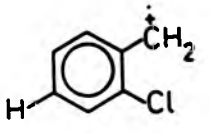
It was decided to prepare the 2,4-dichlorobenzyl alkyl ethers using the Williamson ether synthesis[155] method, first preparing the 2,4-dichlorobenzyl alcohol by reduction of the commercially available 2,4-dichlorobenzoic acid and subsequently reacting this with the required bromoalkane.

The large scale reduction of 2,4-dichlorobenzoic acid described in Section 2.2.2, page 51, proved unsuccessful and a complex mixture of reaction products was obtained. This probably resulted from the vigorous conditions used for the reaction, in particular the use of lithium aluminium hydride. Hydride ions from the lithium aluminium hydride may have undergone nucleophilic substitution into the aromatic ring, replacing one of the chlorine atoms. It is also possible that during the reduction process both the ester and alcohol were formed again resulting in a complex mixture of reaction products.

Confirmation of the hydride substitution into the ring was obtained by GC-MS studies of the reaction products described in Section 3.1.1 (the octadecyl, tetradecyl and dodecyl ether prepared from the reduced 2,4-dichlorobenzoic acid). The gas chromatogram indicates the presence of impurity peaks eg. at 21.99 min for the dodecyl ethers and the mass spectrum shows ions at m/z 142 and 125 which may correspond to the structures shown below:

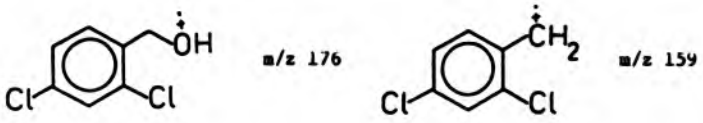


m/z 142



m/z 125

It would be expected that the dichlorobenzyl system would give characteristic mass spectral fragments at m/z 159 and 176 corresponding to the structural fragments shown below:



The mass spectral characteristics are discussed further in Section 4.4, page 173.

As a result this method of preparation of the ethers was not considered suitable and since the 2,4-dichlorobenzyl alcohol was readily available although at a slightly higher price than the dichlorobenzoic acid, the above approach was not pursued.

4.2.3 Synthesis of 2,4-Dichlorobenzyl Alkyl Ethers

The general reaction conditions used are shown in Figure 4.2.

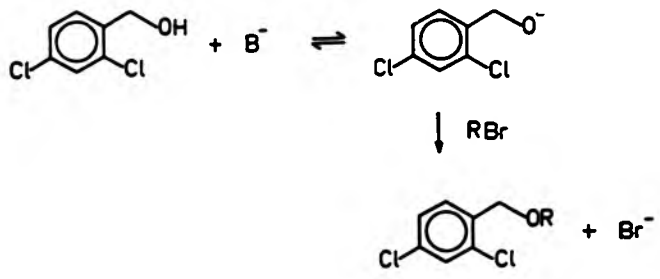


FIGURE 4.2 General reaction conditions for preparation of 2,4-dichlorobenzyl alkyl ethers.

Several series of reaction conditions were examined varying in the base (B^-) and solvent employed. The most suitable solvent was found to be dimethylformamide (DMF), a dipolar aprotic solvent. Early experiments indicated that diethyl ether and tetrahydrofuran were insufficiently polar to allow the reaction to proceed efficiently. These solvents may be unable to stabilise the anion shown in Figure 4.2 whereas the more polar DMF can solvate and stabilise this species.

The principle limitation with the use of dimethylformamide was the difficulty in ensuring that the solvent was 'dry' since the presence of water caused destruction of the base. Distillation from calcium hydride under reduced pressure was sufficient to remove the water. However, dimethylformamide required storage over calcium hydride and the storage time was limited to a maximum of seven days before the DMF required further purification.

The most efficient bases examined were sodium hydride and potassium tert-butoxide, the potassium carbonate proved unsuccessful. With sodium hydride as the base, the reaction proved relatively straightforward and the desired compounds were the principle products. These were readily purified using standard methods which will be discussed in Section 4.2.4. 'Dry' sodium hydride was used since the oil suspension was more difficult to handle and contained a higher risk of side reactions or contamination of the products. The proposed reaction scheme is as shown in Figure 4.2 where B^- = (sodium)hydride.

Side products of the above reaction (Fig 4.2) were not investigated and the precise yields were not calculated since the final purification procedure necessarily caused large losses of material. The yields, however, of the crude reaction product were in the range 30%-70%.

The major limitations in using sodium hydride as the base were:

a) the overnight reaction time and b) the possibility that side reactions may occur when using a powerful nucleophilic base, in particular hydride substitution into the aromatic ring. Therefore, a non-nucleophilic base was preferred and potassium tert-butoxide was chosen. The proposed reaction scheme is as shown in Figure 4.2 where B^- = (potassium) tert-butoxide.

The best yields from this reaction (approximately 3g of product, 50-70%) were achieved using the conditions stated above. The overall reaction time was shorter using potassium tert-butoxide than with sodium hydride and the 'work-up' procedure was simpler since it did not require removal of the DMF by distillation. The overall yields from the two reactions did not appear to differ but as stated earlier no detailed measurement was made. Thus the shorter reaction time, the ease of use of the potassium tert-butoxide and the reduced possibility of nucleophilic substitution into the ring made potassium tert-butoxide the preferred base.

4.2.4 Purification of 2,4-Dichlorobenzyl Alkyl Ethers

Before the DCBEs could be used as either internal standards or retention index markers, they required purification to remove any reaction side products or traces of solvent. This ensured that the DCBEs were free of any possible contaminants which could interfere in the gas chromatographic separation. The reaction products were initially purified using chromatographic techniques, followed by either distillation or sublimation under reduced pressure.

The chromatographic techniques used were either the 'Chromatotron' or a short silica column. The 'Chromatotron' was a preparative centrifugally accelerated radial thin layer chromatograph with the separation ocurring in a thin layer of absorbent on a rotor plate which

was driven by a motor at constant speed. A diagram of the Chromatotron is shown in Figure 4.3.

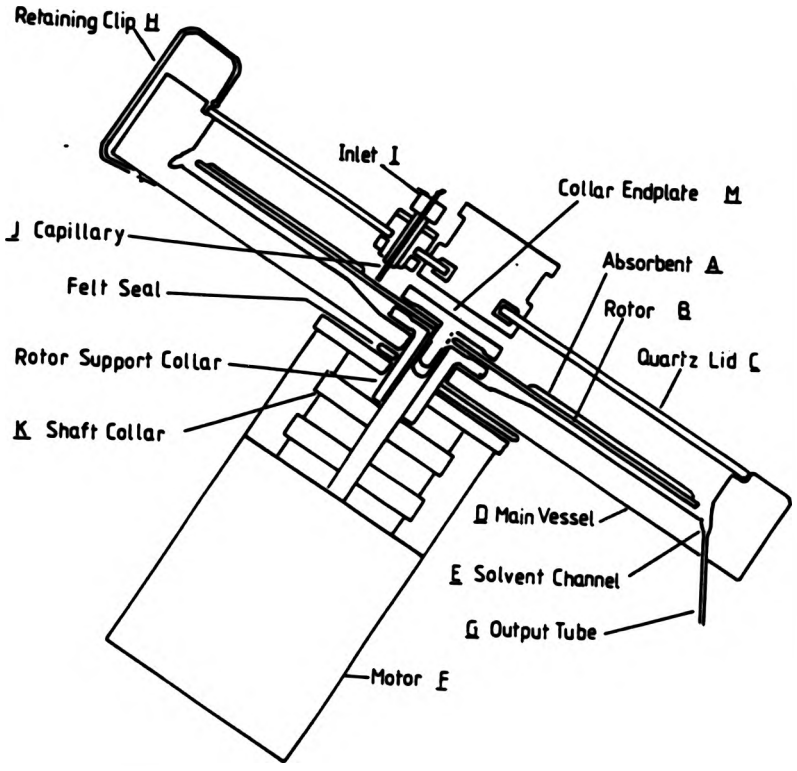


FIGURE 4.3 Schematic diagram of Chromatotron.

Solutions of the reaction products were applied to the absorbant by the inlet I and the capillary tube J. Elution by the solvent formed concentric bands of separated substances which could then leave the edge of the rotor, together with the solvent, by means of the channel E. The rotor was covered with a quartz lid which was transparent to ultra-violet

light, and thus allowed the detection of ultra-violet absorbing compounds. A peristaltic pump provided a variable solvent flow for solvent addition and sample introduction.

The principle limitation in the use of the 'Chromatotron' was the mass of the sample which could be separated. The rotor plate with the maximum absorbent layer (4mm) could separate sample loads of up to 1.5g whereas the mass of reaction product was generally between 2g and 5g. Another limitation with this technique was that samples required a preliminary 'clean-up' step, using a short silica column, to remove any polar impurities which could be irreversibly absorbed onto the rotor. As a result, it was decided to use a silica column alone to purify the DCBEs.

The advantages with these columns[158] is that they allow large scale separations and no preliminary 'clean-up' step is required. For the DCBE purification 20g of silica was used for each gram of reaction product and the solvent system was either petroleum ether/ethyl acetate or hexane/ethyl acetate. Hexane was the preferred solvent since it could be obtained in high purity (HPLC grade) and required no further purification, thus reducing the possibility of contamination by solvent residues.

The final step in the purification procedure was either distillation or sublimation under vacuum. This procedure removed volatile impurities including traces of solvent and any particulate impurities, for example silica which remained in the distillation vessel. Using these procedures, the DCBEs had a purity of 99.99% when examined by GC-MS.

Thus it may be seen that DCBEs fulfilled the criteria (Section 1.6) of being relatively easy to prepare and purify. Furthermore, since the DCBEs were either colourless liquids or white solids they were also easy

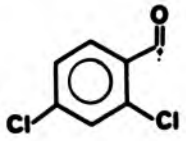
to handle.

4.3 STABILITY OF 2,4-DICHLOROBENZYL ALKYL ETHERS

Another of the criteria stated in Chapter 1 was that the chosen compounds should be thermally and photochemically stable and have a reasonable 'shelf life' of at least two years. Tests were therefore performed on the pure compounds and solution of compounds to determine their stability over time and to study treatment methods used in the Freshwater Fisheries Laboratory.

4.3.1 Stability of Pure Compounds

In general, ethers are a very stable group of compounds. Their only major reactions are auto-oxidation forming the hydroperoxide which can then undergo further reaction[159] and decomposition under acidic conditions. The DCBEs were shown to undergo decomposition when stored in air at room temperature to form the aldehydes. The stability trials described in Section 2.7.2 indicated that the ethers underwent no decomposition when stored under an inert atmosphere (argon) or at low temperatures (-5°C) but did decompose in air at room temperature. The method chosen for washing the storage vessels had no effect. On examination by GC-MS, the principle degradation product was the aldehyde with a characteristic mass spectral fragment at m/z 173. The possible structure of this ion is shown below



m/z 173

It is possible that the esters or acids may also have been formed but

ions corresponding to these compounds did not appear in the mass spectrum.

The results suggest that those ethers which are solid at room temperature were less sensitive to the auto-oxidation process than those which are liquid at room temperature. The physical state may affect the sensitivity of the compound through its influence on the kinetics of the process. For the oxygen to react it must react first react with the molecules on the surface layer of both the solid and liquid ether and then permeate through this layer to react further. This process is likely to be much faster in the liquids than the solids.

4.3.2 Stability of Solutions of 2,4-Dichlorobenzyl Alkyl Ethers (DCBEs)

The 1000mg l^{-1} of the pentyl ether (described in Section 2.7.1, page 63) remained stable over a period of nine months under a range of conditions with no sign of decomposition. The limitation with this method of storage was the difficulty in ensuring a constant concentration over a long period of time. Dilute solutions of the DCBEs (0.5mg l^{-1}) remained stable over a period of eighteen months.

4.3.3 Stability of DCBEs to laboratory methods used for the Chemical Clean-up of Liquid Extracts

The DCBEs were stable to derivatisation by methyl iodide and alkaline hydrolysis but decomposed on treatment with concentrated sulphuric acid. This latter reaction is common for all benzyl ethers since they are weak bases and are converted to highly reactive salts by concentrated acids.[159] The DCBEs were also stable to elution with hexane through an alumina column but eluted in the second fraction collected.

Thus it may be seen that the DCBEs suffered from some problems of stability inherent in their chemical nature. However, these problems

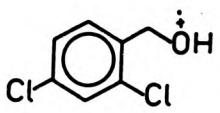
could be largely overcome by a) storing them either as solutions or b) storing them in an inert atmosphere at reduced temperatures. Of these possibilities b) is preferred since problems may be encountered in maintaining constant solution concentrations. The results also indicate that it would not be practicable to add a solution of the DCBEs to environmental samples prior to 'clean-up' since they are decomposed by acid and all elute in one fraction from the alumina column.

4.4 MASS SPECTRA OF DCBEs

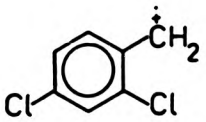
Another criterion stated in Chapter 1 was that the DCBEs should have characteristic ions in the mass spectrum to allow single ion monitoring and easy identification by GC-MS. The most abundant ions in the mass spectrum for each of the DCBEs are shown in Table 3.1 on page 89 and a characteristic spectrum is shown in Figure 3.8 on page 90 . The spectra were recorded using the GC-MS with an ionization energy of 70 electron volts and helium as the carrier gas.

The series of ions m/z 55, 69, 83, 97 and 111 is characteristic of alkyl ethers and corresponds to the general structure $C_nH_{2n-1}CO$. This pattern was particularly observed with nonyl-hexadecyl ethers. Another characteristic pattern present in the spectra was m/z 57, 71, 85 which is a feature of alkyl chains and corresponds to the progressive loss of a methylene group. The general structure for these fragments is C_nH_{2n+1}.

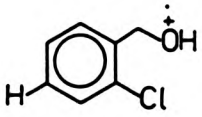
The most characteristic fragments of the 2,4-dichlorobenzyl system were those ions occurring at m/z 176, 159, 141 and 125 which result from the chlorinated benzyl nucleus. The possible structures corresponding to these fragments are shown below:



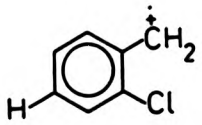
m/z 176



m/z 159



m/z 141



m/z 125

The above ions all represent the masses obtained by considering the ^{35}Cl isotope. In all cases the mass spectra showed the characteristic pattern expected from either a dichlorosubstituted or a monochloro-

substituted compound.

It was also possible to observe ions resulting from the cleavage of the alkyl ether fragment including m/z 143 from the nonyl ether corresponding to $C_9H_{19}O$, m/z 157 from the decyl ether corresponding to $C_{10}H_{21}O$ and m/z 171 from the undecyl ether. In most cases the molecular ion was difficult to observe for the DCBEs and thus the most suitable ions for single ion monitoring are those at m/z 159 and m/z 161, which are characteristic of the chlorinated benzylic system. Thus the DCBEs fulfil the criteria for characteristic mass spectral ions.

4.5 GAS CHROMATOGRAPHIC ANALYSIS OF DCBEs AND CALCULATION OF RETENTION INDICES

The most important criteria the DCBEs should fulfil are that they should be compatible with gas chromatographic analysis, the detectors, linear temperature programming and that they should cover the necessary range of retention times required for environmental analysis (ie from 100 sec to 4000 sec). The results indicate that the DCBEs were compatible with GC-MS, GC-FID and GC-ECD. They also covered the desired range of retention times and were suitable for linear temperature programming. There was also no evidence that the DCBEs were present in any environmental sample. The final factor to examine therefore was the calculation of retention indices for the DCBEs and their use in the qualitative and quantitative examination of environmental samples.

4.5.1 Approach to the Calculation of Retention Indices

Although the n-alkanes are the definitive retention index standards, in theory, any homologous series of compounds should be suitable for use as secondary standards, providing that the logarithm of the retention time is a linear function when plotted against the carbon number of the

standard or that the retention indices of the series are calculated directly using the n-alkanes as primary standards.[42] It was the latter approach which was chosen for this work with the n-alkanes acting as the primary standards with fixed retention index values. The homologous series of 2,4-dichlorobenzyl ethers acted as secondary standards, their retention indices being calculated directly from the n-alkane retention indices using the respective retention times.

The above approach is valid for any available homologous series and it is particularly important in linear temperature programmed gas chromatography where the calculated retention indices are not formal Kovats' indices which are measured under isothermal conditions but instead are subject to the additional parameter of temperature programming. Direct comparisons with the n-alkanes chromatographed under the same conditions allows some account to be taken of this variable.

In linear temperature programming the relationship developed by Van den Dool and Kratz[54] described in Chapter 1, page 22, is important, and allows the calculation of retention indices from elution temperatures again with reference to the n-alkanes. The importance of n-alkanes as primary standards in these calculations is dependent on the extensive studies of their thermodynamic behaviour in gas chromatography. The n-alkanes are the only compounds for which a rigorous thermodynamic investigation of their gas chromatographic behaviour is possible,[76] since the only forces between them and the non-polar stationary phases are London dispersion forces and van der Waals forces and their elution order is dependent only on their boiling point.

4.5.2 Relationships Used in the Calculation of Retention Indices

Kovats[44] developed the relationship shown in equation 6 on page 20

to calculate retention indices in packed columns under isothermal conditions.

Retention time is now used almost exclusively rather than the retention volume and as stated earlier in Section 1.4.2, page 22, Van den Dool and Kratz[54] modified the relationship to use elution temperatures.

The relationship shown in equation 1 is dependent on the use of discrete values of the retention volume, time or temperature and thus the calculation of the retention index of an unknown or a series of unknowns is based on a series of such calculations. The accuracy of these calculations is based on the accuracy of the retention parameter measurement and the long term precision is dependent on the reproducibility of the retention parameters. An alternative approach for the calculation of retention indices, proposed by Ballschmiter[68] is described below.

Since the homologous series of n-alkanes is used as the primary standard for the relationship of the logaritha of net retention time versus the carbon number of the alkane, the relationship can be written such that the logaritha of the retention time of a homologue is expressed as a function of the carbon number.

Over a series of three homologues the relationship can be regarded as a linear function, while over a larger number of homologues a power expansion can be used to describe the experimental results. This non-linear approximation by a power expansion allows interpolation and extrapolation of retention indices over a wide range of homologues. This approach was adopted in the calculation of retention indices of the DCBEs in this work and it had the advantage of allowing the calculation of retention indices without knowing column 'dead time'. The column 'dead time' is a measurement of the time taken for an unretained compound

to traverse the column. Since the value is influenced by column volume and the amount of stationary phase present, which are greater in packed that in open tubular columns, the column 'dead time' must be included in calculating retention indices. Thus, the total retention time in packed columns may be regarded as the sum of two factors, the dead time, which is dependent on the system flow rate as well as the void volume of the column and the adjusted retention time which is characteristic of the separation process. However, in open tubular columns the magnitude of the dead time is small and only dependent on the flow rate of the mobile phase and hence should add as a constant factor in calculations. This allows a comparison of absolute retention times rather than adjusted retention times. Recent developments in equipment and column technology have made it possible to record retention data on an absolute basis over prolonged periods of time.[63] As a result, the approach in this work used absolute retention times rather than the logarithm of retention times in retention index calculations, since this required fewer manipulations in the treatment of chromatographic data.

The calculation of the retention indices for the DCBEs involved the measurement of the retention times of the homologous series of n-alkanes and the development of a mathematical relationship describing this chromatographic behaviour. The DCBE retention times were then measured and the retention indices calculated by interpolation using the above relationship. The n-alkanes and DCBEs were co-injected to minimise errors in time measurement and any possible system variations.

In the Freshwater Fisheries Laboratory two possible mathematical methods were considered for the calculation of retention indices for the DCBEs, a polynomial function and a cubic spline function. Initially, a fifth order polynomial was used and the results are described in Section

3.3, page 96. However, in consultation with Dr H Buchert, a cubic spline function[160] was developed within the laboratory[176] which allowed the calculation of results directly from a 'Chromatochart' programme as described in Section 2.9, page 70. The results calculated for the DCBEs and the subsequent application of these values were the first time the above approach had been used. Consequently, there is no available literature with which to compare the results and the method. The only method for assessing this technique is by applying the results to a 'real' problem, in this case the measurement of organochlorine residues in fish oils. It is impossible to compare the retention index values of the DCBEs with literature studies for two reasons: a) The DCBE retention indices were not measured under isothermal conditions and hence are not formal Kovats' indices; b) Most retention indices discussed in the chemical literature are from simple or branched alkanes and the retention indices have been measured using isothermal conditions and packed columns.

4.6 CALCULATED RETENTION INDEX VALUES OF DCBEs

In all cases the calculated retention index values were rounded to the nearest whole number, since the estimated errors in the calculation were greater than 0.5 retention index units (RIU) and facilitated data inputing to the computer.

The preliminary calculations of the retention indices of the DCBEs (the values are shown in Sections 3.3.1 and 3.3.2, pages 96-98) were performed using an incomplete DCBE series. Two standard series were used for the calculations, the n-alkyl trichloroacetates (ATAs) and the n-alkanes. Only those values calculated using the n-alkanes will be discussed since they may be compared with the later work using the complete DCBE series. The ATAs were also assigned arbitrary values rounded to the nearest 100 RIU; For example, 1500 and 1600 for the carbon numbers 7 and 8 respectively and hence the retention indices for the DCBEs could not be related back to the primary standards, the n-alkanes.

The results described in Sections 3.3.3 and 3.3.4 were also calculated using the mass spectrometer and single injections of each solution. As a result a study of the reproducibility of the method was impossible.

The retention indices of the complete DCBE series from the ethyl to the hexadecyl ether (the mean retention times and retention indices are shown in Tables 3.10-3.13, pages 106-115) were calculated using the cubic spline function and the analysis was performed using a gas chromatograph fitted with a splitless injector and a flame ionization detector. An auto-sampler was used to minimize injection errors due to different injection techniques or operators and overall operation, including data collection and processing, was controlled by an Apple IIe microcomputer.

Over the measured range of 1000-3000 RIU the reproducibility of the retention indices was good with the values showing a maximum variation of ±2 RIU for each set of conditions shown. Unresolved alkane and DCBE peaks usually resulted in a broader peak and the calculated retention indices showed a greater variation than with the resolved peaks. Although it would have been preferable to have both series completely resolved, this proved impossible to achieve given the retention time separation between the members of each homologous series. The reproducibility of the retention indices was therefore dependent, not only on the treatment of the data but also on the raw data itself which was dictated by the nature of the chromatographic separation. The results indicated that good reproducibility can be achieved provided an accurate timing mechanism and data treatment was used.

In the following sections consideration will be given to the range of values obtained using different stationary phases and chromatographic conditions.

4.6.1 Retention Indices of DCBEs on a CPSil5-CB Column

The effect of different starting temperatures and programme rates was greatest for the first members of the DCBE series ie. the ethyl-heptyl ethers. A summary of the values for the ethyl, propyl and butyl ethers is shown in Table 4.1 below.

The greatest difference observed was between the values calculated using a starting temperature of 80°C and 3 deg min^{-1} and those calculated using a starting temperature of 120°C and a programme rate of 7 deg min^{-1}, for example 18 RIU for the propyl ether. The results calculated using a programme rate of 7 deg min^{-1} must be considered the

Table 4.1 Variation in retention indices for ethyl-propyl ethers on a CPSil5-CB using different starting temperatures and temperature programme rates.

Starting Temperature (^{o}C)	Programme Rate ($^{o}C\ min^{-1}$)	Retention Index Ethyl	Propyl	Butyl
80	3	1363	1455	1556
100	ditto	1368	1460	1559
120	ditto	1373	1473	1569
80	5	1365	1458	1559
100	ditto	1370	1461	1561
120	ditto	1373	1472	1569
80	7	1366	1460	1562
100	ditto	1371	1463	1564
120	ditto	1375	1473	1570

least accurate of the results. The fast programme rate caused a reduction in resolution between the n-alkanes and the DCBEs as indicated by the mean retention times shown in Table 3.10 on pages 106-108. Since these conditions are rarely used for environmental analysis using open tubular gas chromatograpny the retention indices will not be included in any future discussion.

With programme rates of 3 deg min^{-1} and 5 deg min^{-1} variations in the retention indices appeared to be a result of the different starting temperatures used and the effect appeared greatest with the ethyl-octyl ethers. A summary of the retention indices for the ethyl-butyl ethers is shown above in Table 4.1.

In changing from a starting temperature of $80^{o}C$ to $100^{o}C$ the greatest variation (2-5 RIU) was observed for the ethyl-hexyl ethers.

Since these compounds have the lowest boiling points, and hence the shortest retention times, they consequently have the shortest period for equilibration between the injector at 275°C and the column. As a consequence, any change in the initial column temperature affects the retention times of these early members more than the later eluting compounds.

It should be noted that these conditions affect not only the DCBEs, but also the n-alkanes which bracket them. Any variation in the retention behaviour of the individual n-alkanes is thus transferred to the DCBEs. Since, by definition, the retention index values of the n-alkanes are fixed, the retention index values of the DCBEs reflect not only the retention characterisation of the DCBEs but also those of the n-alkanes.

The retention index values of the DCBEs eluting after the hexyl ether did not vary by more than 2 RIU. This probably reflects the reduction in the effect of the starting temperature. The increasing effect of the programme rate reduced the resolution between the DCBEs and alkanes when changing from a programme rate of 3 deg min^{-1} to 5 deg min^{-1}.

4.6.2 Retention Indices of DCBEs on a CPSil8-CB Column

The retention times and retention indices of the DCBEs (Tables 3.12 and 3.13. pages 111-115) showed similar trends using programme rates of 3 deg min^{-1} and 7 deg min^{-1} to those observed on the CPSil5-CB column, ie. with increasing starting temperature the retention times decreased for the series. However, these results obtained using a temperature programme rate of 5 deg min^{-1}, showed no variation in retention time with increasing starting temperature and hence no variation in retention index. This suggests that instead of the chromatographic conditions

changing they remained constant. There was insufficient time available to repeat the analysis and it is suggested that this part of the experiment be repeated in any future work. The results for the retention indices obtained using temperature programme rates of 3 deg min^{-1} and 7 deg min^{-1} show a similar trend to those recorded using the CPSil5-CB column.

4.6.3 Variation of Retention Index Values of DCBEs on CPSil5-CB and CPSil8-CB Columns

The open tubular columns used in the analysis were supplied by Chrompack UK and were constructed of fused silica with an internal diameter of 0.22mm and were 25m in length. The stationary phase had a film thickness of 0.12μm and was chemically bonded. The polarity of the stationary phase was denoted by the CP number which was defined by Chrompack.[161] The CPSil5-CB column was a chemically bonded 100% dimethylpolysiloxane stationary phase whereas the CPSil8-CB column was a chemically bonded 5% phenyl/95% methylpolysiloxane phase, thus the CPSil8-CB column was more polar. It is useful to compare the retention indices of the DCBEs on these two columns since the only variation is the stationary phase polarity, and the results are summarised in Table 4.2.

The differences in the retention indices of the DCBEs on the two columns not only reflect the difference in retention for the DCBEs but also those of the n-alkanes as stated previously on page 183.

In analyses where the two series under consideration are very similar, both chemically and physically, it would be expected that the retention indices would also be similar when compared on different polarity columns. However, since the alkanes and DCBEs differ, their retention with respect to each other would alter on changing column polarity. Again this change in relative retention would be reflected

Table4.2 Differences in RI for DCBEs on CPS115CB and CPS118CB at 3 deg min^{-1}

	Starting temperature					
	80°C		100°C		120°C	
	CPS115CB	CPS118CB	CPS115CB	CPS118CB	CPS115CB	CPS118CB
D2	1363	1382	1368	1388	1373	1400
D3	1455	1475	1460	1478	1473	1485
D4	1556	1576	1559	1579	1569	1585
D5	1657	1679	1659	1681	1660	1685
D6	1759	1780	1760	1782	1762	1785
D7	1861	1884	1862	1885	1866	1887
D8	1963	1987	1965	1987	1966	1989
D9	2067	2091	2067	2091	2068	2092
D10	2170	2195	2171	2195	2171	2196
D11	2275	2299	2275	2299	2275	2300
D12	2380	2404	2379	2405	2379	2405
D13	2484	2509	2483	2510	2483	2510
D14	2589	2615	2588	2615	2588	2615
D15	2694	2719	2694	2719	2694	2719
D16	2800	2825	2800	2825	2800	2825

solely in the DCBE retention indices.

For any specific compound, its partition coefficient, which is an indication of its retention characteristics, is determined by the magnitude of the intermolecular forces involved in the interaction with the stationary phase. With the n-alkanes these intermolecular forces are exclusively London dispersion forces which are essentially additive and increase with increasing molecular weight. However, with the introduction of heteroatoms and multiple bonds, affecting the π systems in molecules, the retention behaviour becomes dependent on the molecular size (volume), its shape and any functionality which may be present. According to Golovnya et al[73] the retention index may be regarded as a universal parameter which describes the sorption and structural properties of the investigated substance. Thus, changing the stationary phase polarity alters the intermolecular parameters between the molecule and stationary phase hence altering the retention index.

Another factor which may be important is the effect of temperature programming. Curvers et al[57] stated that temperature programmed retention indices are affected by the initial temperature, the programming rate, the carrier gas velocity and the temperature dependence of the partition coefficient. Since this final factor will differ from the DCBEs to the n-alkanes, changing column polarity will change this factor again altering the retention characteristics.

4.6.4 Observed Incremental Values of Retention Indices of DCBEs

One of the rules proposed by Kovats relating to retention indices[49] stated that the difference in retention indices between two members of a homologous series should be a constant number approaching 100. Since Kovats proposed the rule for isothermal gas chromatography it may be interesting to examine the increments between the DCBEs under

the effect of temperature programming with a constantly changing temperature dependent parameter ie. the partition coefficient. Diagrams of the deviation from 100 RIU against carbon number, for a programme rate of 3 deg min^{-1} using CPSil5-CB and CPSil8-CB columns, are shown in Figures 4.4 and 4.5. The greatest deviation from 100 RIU was apparent in the early members of the series (ethyl to hexyl ethers) after which the increment was 102-106 RIU.

Although Kovats stated that the increment should be constant, it has been widely reported that deviations occur[71] and the evidence was reviewed by Smith, Haken and Wainwright.[72] It has also been shown that the retention index contributions per methylene unit in a homologous series deviates from 100 for the lower members and it has been stated that Kovats' rule only applies to the higher members of the homologous series.[73]

According to Golovnya,[75,162] the retention index, as defined by Kovats, is a universal relative parameter describing the sorption and structural properties of an investigated substance. Golovnya postulated that the non-linear variation observed for a homologous series was due to the energy contributions of the first methylene groups being less than for the second, and that the further the position of the methylene group from any functional group, the less the effect of the functional group. For the DCBEs, in which the functional group may be regarded as the benzyl ether nucleus and hence there would be a significant deviation from 100 for the change from the methyl to ethyl ether and the effect would continue to the butyl or pentyl ether, and was observed in the results. It is possible that the effect of the benzylic ether functionality may be shown by nmr studies of the DCBEs. The deviation from 100 RIU increments may also be due to the effect of linear

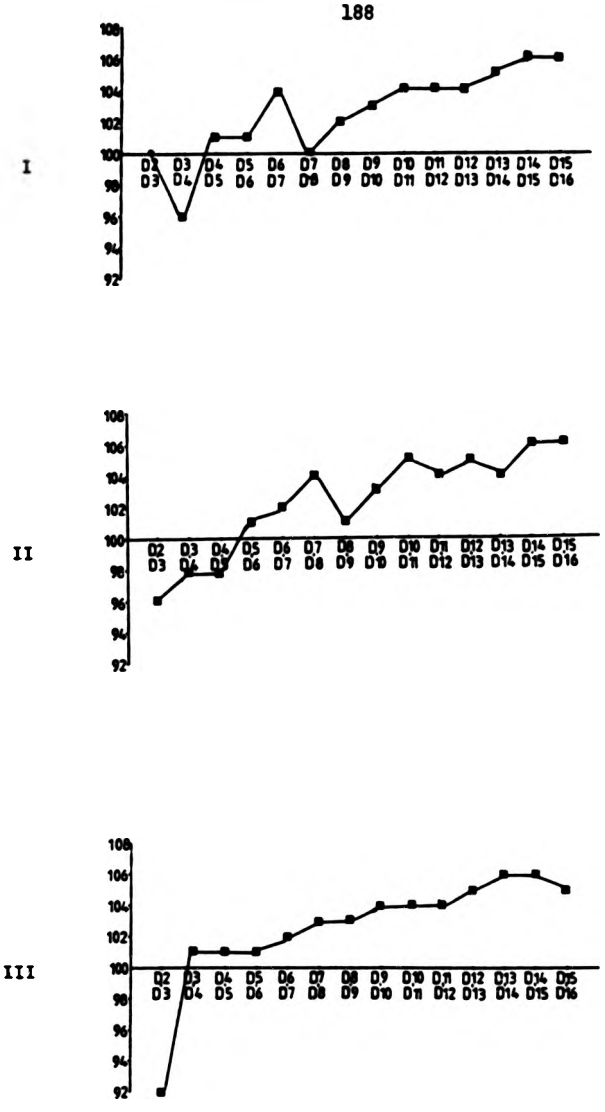


FIGURE 4.4 Diagrams of increment values of DCBEs for programme rate of 3 deg min^{-1} on CPS115-CB column. I) Starting temp 80°C
II) Starting temp 100°C
III) Starting temp 120°C

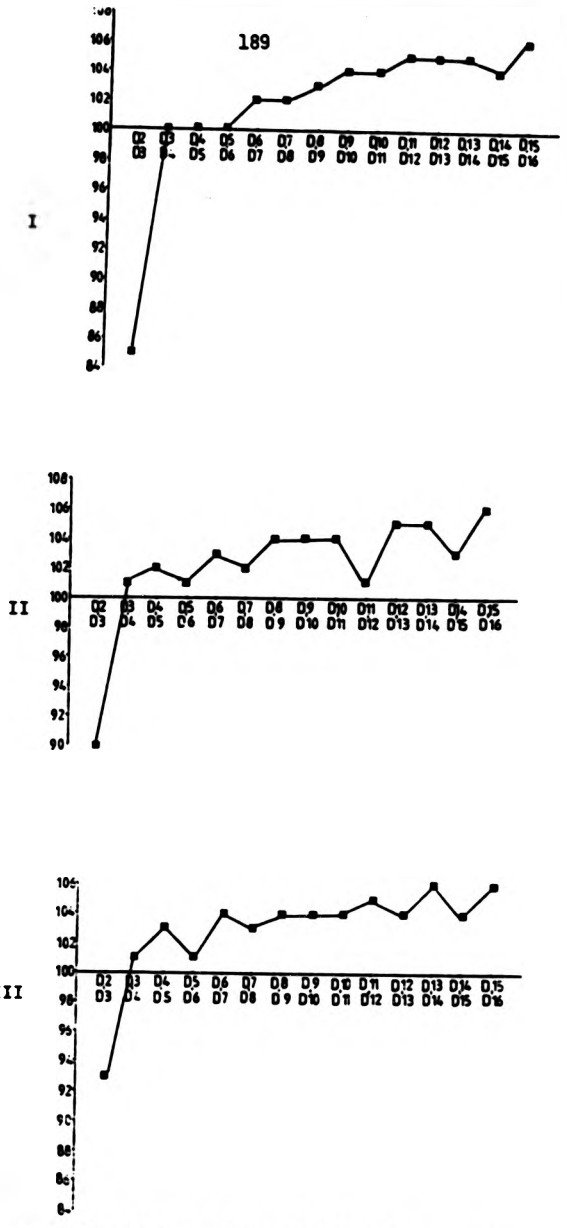


FIGURE 4.5 Diagrams of increment values of DCBEs for programme rate of 3 deg min^{-1} on CPSil8-CB column. I) Starting temp 80°C

II) Starting temp 100°C

III) Starting temp 120°C

temperature programming and different starting temperatures. As already stated, the DCBE retention indices are not formal Kovats indices and to consider the incremental values in detail it would be necessary to examine the DCBEs under isothermal conditions on non-polar stationary phases to minimise the effect of external parameters, and compare these results with the temperature programmed results.

4.7 CALCULATION OF RETENTION INDICES OF ORGANOCHLORINES ON DIFFERENT COLUMNS AND USING DIFFERENT GC CONDITIONS

The retention indices of the organochlorine standard compounds are as shown in Tables 3.15, 3.17, 3.19 and 3.21 of Chapter 3 and the procedure used for calculation was similar to that already described. The compounds chosen for examination were those which were routinely screened for by the Freshwater Fisheries Laboratory and consisted of a range of polychlorinated biphenyls and organochlorine pesticides and their metabolites. In this work the retention indices were examined using on-column injection. Subsequent work by Freshwater Fisheries staff[177] showed no difference between this injector and splitless injectors. The structures of these compounds are shown in Appendix 1.

The retention indices were calculated using the DCBEs as fixed standards using different gas chromatographic conditions of starting temperature and programme rate. It was also decided to examine the effect of internal standards on the retention index values and whether it was possible to use a limited number of the DCBEs rather than the complete series. It was hoped that instead of having a separate DCBE calibration run for each sample series it would be possible to 'spike' the environmental samples with six-eight DCBEs. This would allow the possibility of using multiple internal standards.

4.7.1 Retention Indices of DCBEs and Organochlorine Standard Compounds on a CPSil5-CB Column using a Temperature Programme of 3 deg min^{-1}

The retention indices are shown in Tables 3.14-3.17. The values of the DCBEs, calculated using the first DCBE injection in each case as the calibration standard, showed a maximum variation of 4 RIU and again this was probably due to the stabilisation of chromatographic conditions as discussed in Section 4.6.1. There was little difference in the results

calculated with or without an internal standard, an internal standard is recommended to ensure the long term precision and accuracy of the results.

The results calculated using the complete and the incomplete DCBE series did vary. This was not large for the starting temperature of 80°C but at 100°C and 120°C the ethyl and propyl ethers showed variations of 38 and 72 RIU respectively. This effect is demonstrated empirically by consideration of Figure 4.6:

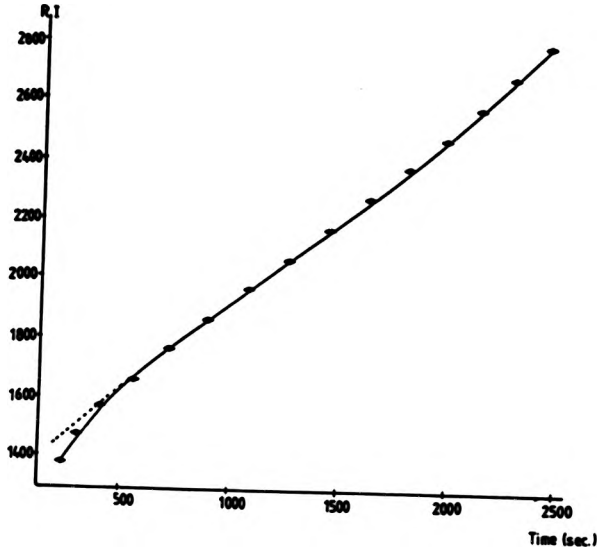


FIGURE 4.6 Diagram showing effect of removing ethyl and propyl ether values on DCBE calibration.

which shows the relationship between retention index and time for a starting temperature of 120°C. The unbroken line shows the results

determined using the complete series of the DCBEs, whereas the broken line shows the effect of removing the ethyl and propyl ethers and extrapolating the other results. Although this is not a quantitative examination, it gives some idea of the effect of extrapolating the function for the early eluting compounds. The effect of removing other members of the DCBE series such as the pentyl, nonyl or undecyl ether was less important since it was still possible to interpolate results from the other ethers. However, the results indicate that at least one DCBE should elute before the first compound of interest in any analysis, allowing accurate retention index calculation and hence compound identification.

The retention indices of the organochlorine compounds showed little variation when the results were calculated using the complete DCBE series or the test series. There was also no significant variation between the results calculated with or without an internal standard within each set of conditions. The maximum variation encountered within each set of conditions was 4 RIU for the initial starting temperature of 120°C.

The retention indices of the organochlorines showed some variation with the conditions used. After the elution of 2,4-DDE in both standard solutions the values did not change by more than 3 RIU but those compounds eluting before this peak showed variations of up to 13 RIU (HCB* and HCH*).

---

* Structure shown in Appendix 1

4.7.2 Retention Indices of DCBEs and Organochlorine Standard Compounds on a CPSil5-CB Column using a Temperature Programme of 5 deg min^{-1}

Using an initial temperature of 80°C, values for the DCBEs shown in Table 3.16, page 124 , showed little variation, irrespective of the choice of internal standard or whether the values were calculated using the complete or incomplete series. However, much larger variations were observed using the starting temperatures of 100°C and 120°C where the differences for the ethyl and propyl ethers differed from their measured values by 16 and 53 RIU respectively. Again this may be explained by having to extrapolate the results from the butyl ethers.

The retention index values of the organochlorine compounds shown in Table 3.17, page 130 , were consistent for both the complete and incomplete series. However, a difference of up to 7 RIU was observed where no internal standards were used for PCB 195. The choice of internal standard did not affect the retention index values for either the S1 or S2 standard solutions.

The change in initial temperature again affected the retention index values especially for those compounds eluting before 2,4-DDE* (eg. 9 RIU for HCB*). Larger variations were observed in changing from 100°C to 120°C (the maximum change was 7 RIU rather than 3 RIU for HCB). An important feature to note in using these chromatographic conditions was the marked decrease in resolution for both standard solutions with pairs of peaks merging (eg. 2,4-DDE* and Endosulphan 1* in the S2 solution and PCB194* and D14 in the S1 solution). As a result, these chromatographic conditions are considered unsuitable for the analysis of environmental samples.

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* Structure shown in Appendix 1.

4.7.3 Retention Indices of DCBEs and Organochlorine Standard Compounds on a CPSil8-CB Column using Temperature Programme of 3 deg min^{-1}

The retention indices of the DCBEs shown in Table 3.18 on pages 132-137 showed similar trends to those observed on the CPSil5-CB column with the results unaffected by the choice of internal standard. Use of the complete or test series of DCBEs had the largest effect on the ethyl and propyl ether values especially between the starting temperatures of 100°C and 120°C, for example at the starting temperature of 100°C the propyl ether showed a variation of 10 RIU from the measured value whereas at 120°C the variation was 22 RIU).

The retention indices of the organochlorines shown in Table 3.19, page 138, also showed similar trends to those already described. However, it was apparent that the resolution on this column was much poorer than the CPSil5-CB column especially for the S2, solution with up to 5 peak pairs merging. This factor limits the use of the retention index system and makes the accurate identification and quantitation of individual peaks almost impossible since both of these are dependent on each compound of interest being a distinct peak in the chromatogram. It is likely that the poor resolution was due either to the difference in stationary phase polarity adversely affecting resolution, or that the column was contaminated. With repeated use of heavily contaminated samples, some of the constituents may be irreversibly absorbed onto the column and hence create active sites. This problem can be overcome by the removal of 20-30cm from the front of the column.

4.7.4 Retention Indices of DCBEs and Organochlorine Standard Compounds on a CPSil8-CB Column using a Temperature Programme of 5 deg min^{-1}

Although the retention indices of the DCBEs described in 3.4.1 did not vary with starting temperature it was possible to use these results to study the retention indices of the organochlorines. Similar trends were observed as described before with the change in starting temperature having the most significant effect on the early eluting compounds and the choice of internal standard having no marked effect. It must be noted that the resolution for the S2 solution at 5 deg min^{-1}, compared to that recorded at 3 deg min^{-1}, was improved and this was probably due to the removal of a piece of column.

These results indicated that it was possible to assign retention indices to all the organochlorine standard compounds using the DCBE standard solutions providing there was satisfactory resolution of all compounds. It should be possible to 'spike' any sample with a number of the DCBEs to act as multiple internal standards although the data-handling programme would require expansion. At the time of this work it was only possible to treat one compound as an internal standard. Ideally those DCBE compounds chosen would bracket the peaks of interest and a regular calibration check (once a day) would be done to test the overall performance of the system.

4.8 GEL PERMEATION CHROMATOGRAPHY

Gel permeation chromatography was introduced by Stalling in 1972[163] as a clean-up procedure in pesticide analysis in which SX-2 biobeads were used with a cyclohexane solvent system. The system is a liquid chromatographic technique permitting the separation of molecules by their molecular size rather than their polarity. The separation effect is based on the restricted diffusion of the dissolved molecules in the pores of the stationary phase. As a result, in gel permeation chromatography, polar and non-polar molecules of a comparable molecular size may be isolated in one step, an effect which cannot be achieved with the same efficiency using other chromatographic techniques such as alumina or silica columns. These methods are based on adsorption and partition phenomena which can only separate non-polar compounds whereas the polar components cannot be eluted with the same solvent system without co-eluting interfering matrix compounds.

The gel permeation technique was automated by Findle and Stalling[164] and was evaluated by Griffitt,[165] who found it more efficient and faster in the analysis of pesticide residues in fats than alternative partitioning techniques. Stalling[172] introduced a system with SX-3 biobeads and a toluene-ethyl acetate solvent system which gave quantitative recoveries of non-ionic chlorinated pesticides and PCBs and which could be used in the 'clean-up' of a wide range of sample types. The method employed in this work was that of Hopper[153] who used SX-3 biobeads with a hexane/dichloromethane solvent system since this system allowed easier evaporation for sample residues, a smaller 'dump' fraction which contained 99% of the lipid residues and a reduced total elution volume while maintaining quantitative recovery of pesticides.

The stationary phase employed was SX-3 biobeads which are composed

of a semi-rigid cross linked polystyrene gel with a particle size of 40-75μm and a fractionation range of up to 2000 amu.[167] The permeation of molecules into the gel structure depends solely on the size ratio of molecules to pores, other factors of separation, such as interaction with the solvent, having little significance.

4.8.1 Fish Oils Under Examination

Crude and refined cod liver and capelin oils were supplied by the Marfleet Refining Company. The organochlorine content was measured in each to determine the effectiveness of the refining process.

The fat in any living fish is free of any objectionable colour, flavour or odour constituents. However, after death, enzymatic, bacterial and oxidative processes begin which alter the chemical and physical properties of the oil. The substances which may affect the quality of the fish oil include free fatty acids, proteins, carbohydrates, phospholipids and products from the auto-oxidation of the oil. The oil must therefore undergo a refining process, the object of which is to remove the above contaminants, in particular the free fatty acids, which are largely unsaturated fatty acids, so that their concentration does not exceed 0.1%.[168] The refining processes employed include treatment with sodium hydroxide to remove soaps (alkali refining), deodourisation using steam towers or superheated steam, low temperature filtration to remove high melting point fractions and bleaching using activated charcoal to remove natural pigments. The methods used have been discussed by Gauglitz et al.[169]

The two types of fish oil examined in this work were a crude and highly refined cod liver oil and a crude and refined capelin oil. The capelin, (Mallotus villous L) is a small fish (20cm) found in the northern regions of the Atlantic and Pacific oceans. They are extremely

abundant in coastal waters and are eaten by almost all fish-eating birds, mammals and fishes of the Arctic seas. They are therefore basic to many of the food chains of the northern seas. Most of the present capelin catch is used for the production of meal and oil. Capelin oil has been widely used in the production of margarines and shortenings after hydrogenisation and refining.[170,171]

Cod (Gadus mordua L) is approximately 120cm in length, and is found throughout the North Atlantic, feeding on a wide range of fish including capelin and herrings. It is an extremely important commercial fish with much of the catch marketed fresh or frozen. Cod liver oil is used for medicinal purposes.[170,171]

4.8.2 Gel Permeation Chromatography of fish oils

The chromatograms shown in Figures 4.7-4.10, pages 200-201, illustrate the major differences between the crude and refined fish oils. In each case the areas of the uv-active peaks were larger in the crude oils rather than the refined oils, and the refined oils showed two distinct uv-active peaks. The overall lipid volume (shown in Table 3.26, page 150) was greater for the crude oils rather than the refined oils. Some overlap with the organochlorine region was observed although the non-volatile residues did not exceed 6mg (Table 3.27) for an injected mass of 400mg.

The relative position of the organochlorine fraction was determined by measuring the peak positions of two representative organochlorine compounds. The compounds chosen were hexachlorobenzene and decachlorobiphenyl since these compounds were representative of the range of molecular sizes encountered. The hexachlorobenzene would be expected to have the larger retention time since it was the smaller molecule.

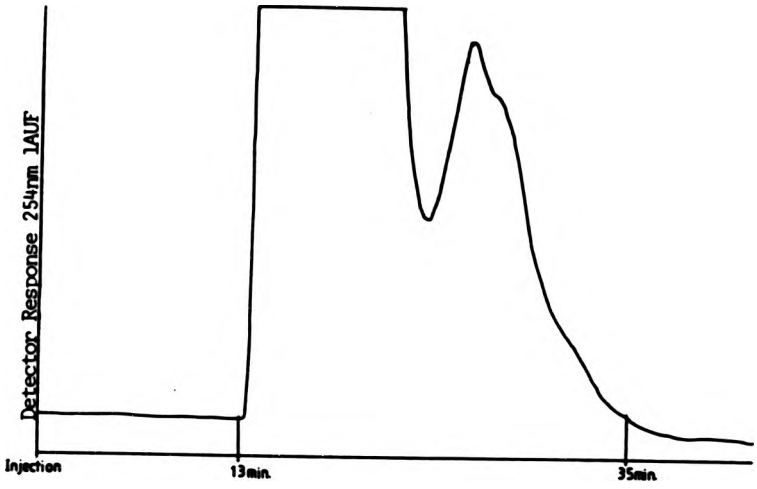


FIGURE 4.7 Gel permeation chromatogram of refined capelin oil.

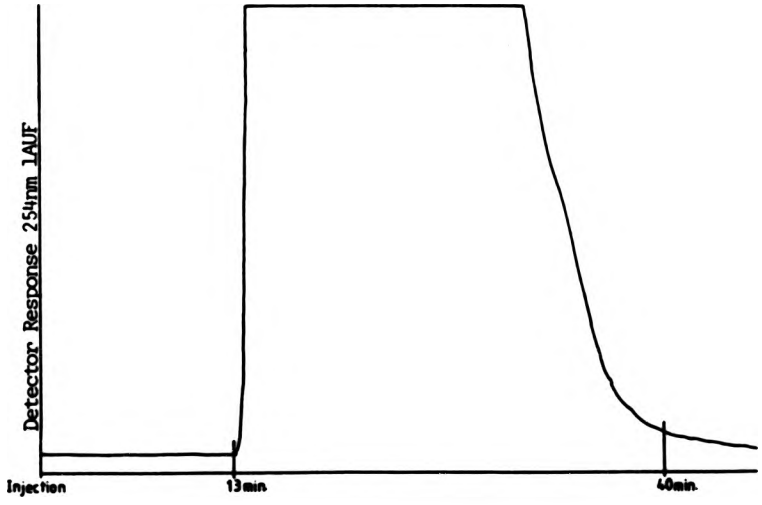


FIGURE 4.8 Gel permeation chromatogram of crude capelin oil.

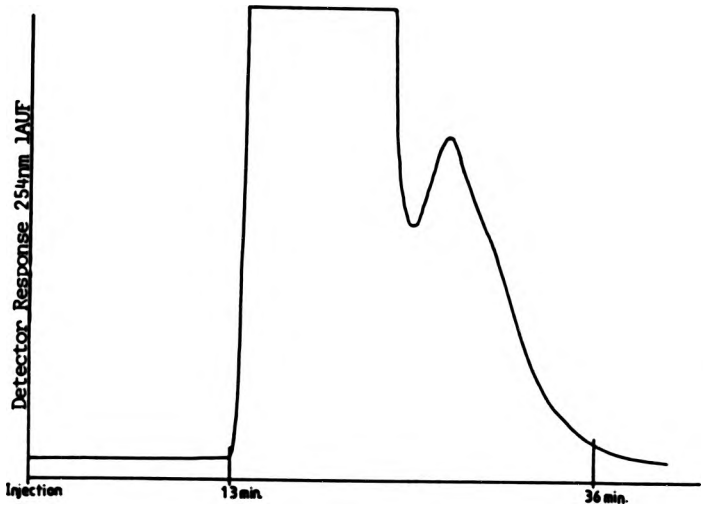


FIGURE 4.9 Gel permeation chromatogram of highly refined cod liver oil.

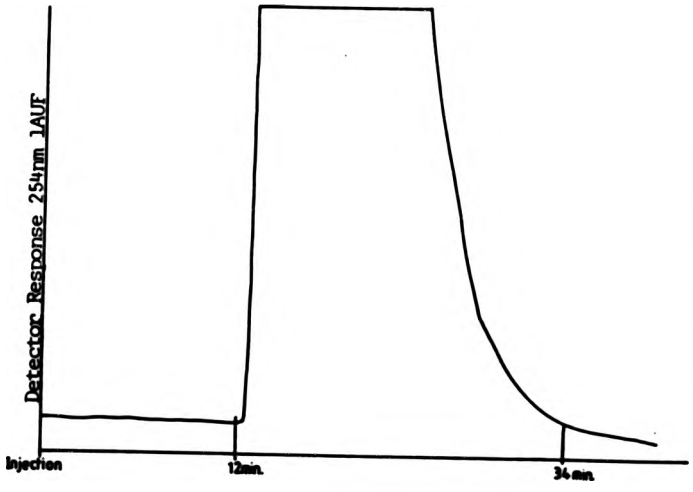


FIGURE 4.10 Gel permeation chromatogram of crude cod liver oil.

As discussed, the gel permeation system separates on the basis of molecular size and thus the larger molecular weight lipids are least retained, therefore allowing their separation from organochlorine residues. However, it was not possible to separate those lipid molecules with a similar molecular size to the organochlorine residues. Although these lipid residues should comprise only 1-2% of the total mass injected (after refining), they must be removed before gas chromatographic analysis to prevent column damage.

After establishing the positions of the organochlorine residues relative to the lipid fraction in each of the fish oils, the mass of non-volatile lipid residue present in this fraction was measured. Samples for GC analysis were collected, in each case 10-15ml before the measured elution of decachlorobiphenyl and 100ml of eluate was collected to ensure the coverage of the entire organochlorine range.

4.8.3 Preparation of Samples for Gas Chromatographic Analysis

The removal of lipid residues remaining after gel permeation chromatography was done using adsorption chromatography on silica and alumina columns. The method used was that of Wells, Cowan and Christie[172] based on the techniques of Holden and Marsden[173] and Wells and Johnstone[152] details of which were given in Section 2.10.12 of Chapter 2. This method has been used to 'clean up' extracts of water, sewage sludge, final effluent, sediment, fish and sea mammal tissue. Any compounds with a labile proton such as phenol, sulphonamides and acids were adsorbed onto the basic alumina allowing the elution of the base/neutral components.

An important feature was to restrict the mass of lipid introduced onto the absorption column to prevent overloading, which could reduce the

resolution and adsorption capacity of the alumina. Wells and Johnstone[152] determined that the mass should be no greater than 50mg and that column overload produced a 'concertina' effect on the elution pattern of the determinants.

The above method may also be used as a group separation, low resolution chromatographic technique in which each of the fish oil samples could be separated into three eluates prior to analysis by gas chromatography. Wells et al[172] showed the elution scheme separated some major isomer mixtures and the scheme for this work is as shown in Figure 4.11 overleaf.

The components present in each of the eluates are shown in Table 4.3. Two of the organochlorine standard compounds were not completely separated into discrete fractions, ie. 2,4-DDE and Heptachlor.

4.8.4 Gas Chromatographic Analysis of Organochlorine Residues in Fish Oils

The retention indices were calculated as described in Sections 2.9.2 and 2.10.14, using a data base developed from the organochlorine retention indices described in Tables 3.15-3.19 on pages 121-138. Further work was done in the Freshwater Fisheries Laboratory to assign values to those peaks which were unresolved in the original work. Although it was possible to determine the retention indices of both the DCBEs and the organochlorine standard solutions with a reproducibility of ±1 RIU it was necessary to impose an 'identification window' of ±3 RIU for the peak identification in unknown samples. Schwartz and coworkers[151] used an 'identification window' of ±1.5 RIU and Albro and Corbett,[137] ±2 RIU. The drawback with using a narrow 'identification

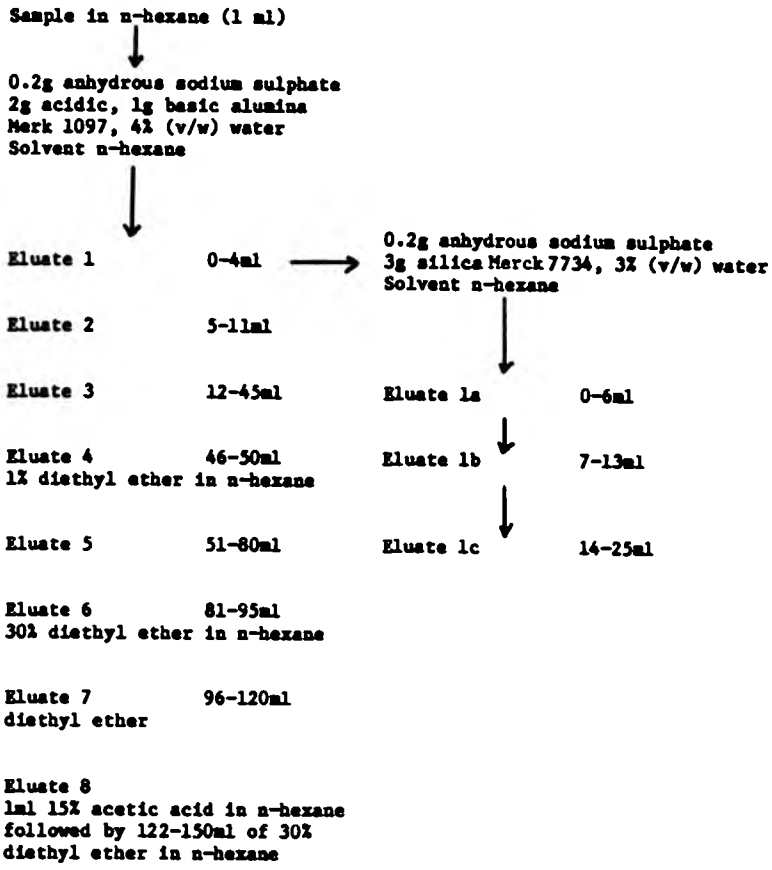


FIGURE 4.11 Elution scheme for isomer pairs of organochlorine compounds.

Table 4.3 The composition of eluates from alumina and silica column separation of organochlorine residues.

Eluate 1A	Eluate 1B	Eluate 2
Chlorobenzenes	Heptachlor 50%	α HCH
Polychlorinated biphenyls	α -chlordene	Y-HCH
Heptachlor 50%	Y-chlordene	α chlordane
Aldrin	2,4 DDE 75%	Y chlordane
2,4' DDE	2,4 DDT	oxychlordane
4,4 DDE	4,4 DDT	Endosulfan -1
Mirex		trans Nonachlor
		2,4' DDD
		4,4' DDD
		Heptachlor epoxide
		Dieldrin
		Endrin
		β HCH
		cis permethrin
		trans permethrin

window' is that in very complex samples the peak position may be affected by the sample matrix and undergo a change in position or may be unresolved from another sample component.[143] These factors alter the position of the peak maximum and thus may be outside the 'identification window'. On the other hand, too large an 'identification window' may result in more than one peak being identified. Thus an 'identification window' of ± 3 RIU will allow for the effect of 'rounding errors' and for some degree of peak movement but it should prevent peak mis-identification since none of the organochlorines has had less than 3 RIU between them when resolved.

Another problem which may be encountered in peak identification is that on any given column more than one compound may have the same retention time. This was overcome by re-analysing the samples or a chosen sample on another column of different polarity since it is unlikely that any two compounds will have identical retention on the two columns. Using this technique the retention indices of peaks in the chromatograms of the fish oils could be determined and identified by comparison with the measured values of organochlorine compounds. The quantity of each component was determined using external standardization and internal standards; the heptyl and tetradecyl ethers for the eluate 1A and the heptyl and dodecyl ether for the eluate 1B+2. The concentration of the organochlorine compounds in the standard solutions were 0.02mg l^{-1} for the analysis using the CPSil5-CB column and 0.02 and 0.1mg l^{-1} for the analysis using the CPSil8-CB column.

The accuracy of the peak quantitation can be assessed by examining the values calculated for the two internal standards in each solution. In each case the concentration of the internal standard in the sample was 12.5 $\mu g kg^{-1}$ and the variation observed was up to 3 $\mu g kg^{-1}$ although it was

generally under 1 μgkg^{-1}. However, with each internal standard covering approximately half the chromatogram the error in the measurement could be regarded as $\pm 0.5\ \mu gkg^{-1}$. This is because the accuracy of chromatographic results is increased by having the internal standard close to the compound of interest as discussed in Section 1.5.3 of Chapter 1, on page 41. As a result those values of organochlorine residues in the fish oils with values of less than 0.5 μgkg^{-1} were not considered within the limits of accuracy.

The results for the four fish oils are as shown in Tables 3.28-3.31, pages 153-162. Only those results in which organochlorines in the fish oils were detected using both columns will be considered (see Discussion on page 206). No detectable organochlorine compounds were found in the highly refined cod liver oil or either of the blank solvent injections, but measurable residues of 4,4-DDE and HCB (0.60 μgkg^{-1} and 0.64 μgkg^{-1} respectively) were detected in the refined capelin oil. The greater concentration recorded using a CPSil5-CB column suggests the presence of an unresolved peak and the results indicate the importance of using two different columns for the identification of peaks.

The concentration of the organochlorine compounds was greater in the crude fish oils than the refined oil. The results recorded on a CPSil8-CB column are shown in Table 3.29 on page 158. In the crude capelin oil 4,4-DDE was present in the highest concentrations 3.29 μgkg^{-1} (the results will be quoted using values obtained for those internal standards which eluted closest to the peak of interest). PCB 153 (1.68 μgkg^{-1}) and HCB (1.14 μgkg^{-1}) were also detected. In the crude cod liver oil PCB 153 (2.06 μgkg^{-1}), 4,4-DDE (2.70 μgkg^{-1}), PCB 138 (2.25 μgkg^{-1}), PCB 118 (1.78 μgkg^{-1}) and PCB 180 (1.03 μgkg^{-1}) were present in the greatest quantities.

Examination of the results for eluate 1B+2 (Table 3.31, page 162) showed no significant residues present in the blank injections or the highly refined cod liver oil. Quantifiable residues of HCH (1.17 μgkg^{-1}), Endosulphanl (0.65 μgkg^{-1}) 2,4-DDE and -Chlordane (0.88 μgkg^{-1} - these peaks were unresolved so the value is a combined one for both compounds) and trans-Nonachlor (0.73 μgkg^{-1}) were present.

The results for the crude oils (shown in Table 3.31 for the CPSil8-CB column) demonstrate quantifiable concentrations of most of the organochlorine compounds in particular 2,4-DDE/ Chlordane (3.12 μgkg^{-1}) and trans-Nonachlor. It is possible that the presence of the two DDE isomers resulted from the breakdown of DDT, as this is a recognised metabolic pathway.[175]

In considering the relative concentrations of contaminants it must be remembered that the capelin oil was a product of the whole fish whereas the cod liver oil was extracted from a specific organ which is responsible for the metabolism of potential toxins. Thus it is not surprising that the organochlorine concentration was higher in this organ than in the rest of the fish. Capelin is also part of the normal cod diet and hence any organochlorines present in these fish would undergo further metabolism in the cod increasing the concentration present. Thus organochlorines may be subject to bio-concentration as they pass up the food chain.

The resolution of the organochlorine standard solutions was poorer on the CPSil8-CB column and thus some pairs of peaks were unresolved. This is a drawback using the S1 and S2 solutions since any small deterioration in column performance results in the merging of peaks. However, this problem is difficult to overcome unless further clean up procedures are introduced, separating the organochlorines into smaller groups.

4.9 SOURCES OF ERROR IN RETENTION INDEX MEASUREMENT

Vernon and Suratman[70] reviewed the possible sources of error in calculating retention indices on packed columns. These include the measurement of column dead time, the concentration of the bracketing alkanes, the sample size and concentration, the support activity and the purity of the stationary phase. Ettre[48] also discussed factors which may cause error and included instrumental variations in temperature and gas flow and inaccuracies in the measurement of retention time.

With the use of open tubular columns, the stationary phase and column purity are much more consistent and there is generally less column activity. New developments in instrumentation, in particular, improved temperature control and the increasing use of computer control, have greatly improved instrument performance. Neu and Zinburg[141] found that accuracy in retention time measurement is the most important factor in retention index calculations and stated that the precision of retention time measurements should be 0.01%.

In this work the reproducibility of the retention times shown in Tables 3.10 and 3.12 indicated that the retention times did not vary by greater than ± 3 seconds for the DCBEs and n-alkanes in analysis times of up to 3500 seconds. Co-injection of the DCBEs and n-alkanes also minimised possible errors in time measurement.

The greatest possible source of error in measuring retention indices for both the DCBEs and organochlorine compounds was the co-elution of peaks either due to poor column performance or the compounds having the same retention. Calculating the retention indices of the DCBEs wider peaks reduced the precision and accuracy of the time measurement and hence the retention indices. For the organochlorines this problem was greater in calculating the retention indices since if two peaks co-eluted

then no specific retention index could be assigned to each individual peak and a double identification was required. This was shown in the analysis of the fish oils where 2,4-DDE and o-Chlordane co-eluted. It must be stated, therefore, that the utility of the retention indices is dependent not only on the calculation of the retention indices but on the overall chromatographic performance. This was demonstrated in the failure of the data acquisition programme to recognise some peaks in chromatograms requiring re-injection of the sample. Therefore, although the retention index system may fulfil all criteria set, the ultimate determining factor is the chromatographic performance.

CHAPTER FIVE

CONCLUSIONS

5. CONCLUSIONS

The objectives of the work were:

1) to prepare a homologous series of compounds which were suitable for use as internal standards and retention index calibration standards;

2) to calculate retention indices for those standards under different gas chromatographic conditions;

3) to use these values in the qualitative and quantitative analysis of environmental samples with the emphasis on organochlorine pesticides and polychlorinated biphenyls.

The compounds chosen were the 2,4-dichlorobenzyl alkyl ethers and their suitability may be assessed by considering how well they fulfilled the criteria stated in Section 1.6.1, page 45. That is;

a) The ethers were readily prepared using the Williamson Ether Synthesis and no major problems were encountered. The preferred base was potassium tert-butoxide because of ease of use, reduced reaction time and lower reaction temperatures. The requisite purity for gas chromatography was achieved using a two stage purification process involving a chromatographic step (a silica column), followed by distillation or sublimation under vacuum. No handling difficulties were encountered as the DCBEs were either colourless liquids or white solids.

b) The DCBEs were easily chromatographed on medium polarity columns and could be detected using GC-FID, GC-ECD and GC-MS. The peak shape was good and the DCBEs covered the required elution range of 500 sec to 4000 sec. They were not present as contaminants in any of the environmental samples studied and sufficient retention time 'windows' were present in the organochlorine standard solutions for

inclusion of up to eight DCBEs, thus allowing the accurate coverage of the entire chromatogram. The DCBEs could also be used in a 'system check' capacity since any degradation in peak shape of the early eluting compounds indicated a deterioration in chromatographic performance requiring either a change of column or removal of the front portion of the column.

c) The DCBEs were stable in solution but underwent oxidative degradation when stored in air at ambient temperatures, thus reducing their shelf life. However, this could be prevented by storing of the compounds under an inert atmosphere such as argon or nitrogen and/or at reduced temperature (below $0^{o}C$). Alternatively, the compounds could be stored in solution form, although this may present difficulties in ensuring constant reproducible standard concentrations over a prolonged period of time. Further work is required to measure the rate of degradation of the compounds and optimize storage conditions.

However, the most important criterion to fulfil was the efficacy of the DCBEs as internal standards and retention index calibrants. The calculation of the retention indices for the DCBEs was dependent on the use of n-alkanes as the primary standards. It was theoretically possible to assign empirical retention index values to the DCBEs, with an increment of 100 RIU for each methylene group without reference to a primary standard. However, it was decided that using primary standards was a more valid approach since this allowed the retention index values of the DCBEs to be directly correlated with those values for n-alkanes, the only compounds for which a rigorous thermodynamic treatment of their gas chromatographic behaviour is available. This approach also allows inter-laboratory calibration of the retention index system with referral

to the n-alkanes.

The method of calculation chosen differed from that described by Kovats[44] since the DCBE retention indices were calculated under temperature programmed conditions rather than isothermal conditions. The method used was also a continuous function rather than the discrete calculation used by Kovats.

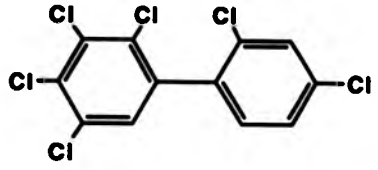
The retention index method for peak identification was combined with internal and external standardization to characterize and quantify environmental samples, in particular a series of four fish oils for which the method proved successful. A further example of use is given in reference 174. An important factor to note is that although the DCBEs could be used in isolation for the identification of peaks they had to be used in conjunction with the external standard solutions to allow accurate quantitation of the contaminants.

It must also be noted that the utility of the DCBEs was not only dependent on their chemical, physical and chromatographic behaviour, but also on the overall performance of the chromatographic system. Should the resolution deteriorate such that it is unable to separate sample peaks, the calibration system could not be used efficiently for either identification or quantitation.

Thus the DCBEs could be used both as retention index calibrants and internal standards in environmental analysis with emphasis on organochlorine and PCB analysis. They are also potentially useful for any gas chromatographic analysis using an electron capture detector, flame ionization detector or mass spectrometer providing a suitable 'retention window' was present. It may also be possible to use isomeric compounds such as the 3,4 isomer or 2,6 isomer to cause a small retention time shift, thus allowing the creation of a new calibration series.

This work should be considered as the initial stage in the development of a calibration system for the analysis of environmental samples. Any further development of the work should consider a more detailed examination of the DCBE retention indices, both in terms of different polarity columns and different chromatographic conditions. The use of these compounds in liquid chromatographic studies should also be examined.

APPENDIX 1



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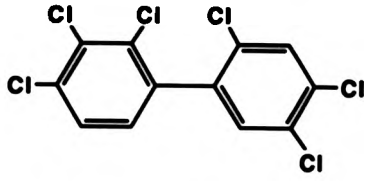

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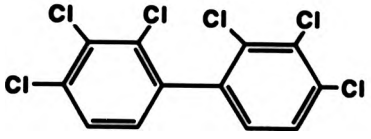

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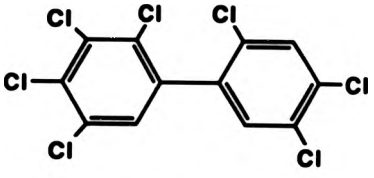

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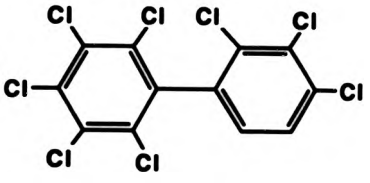

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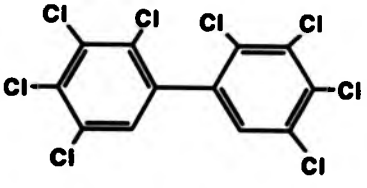

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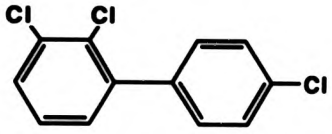

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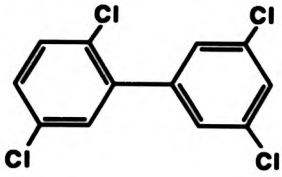

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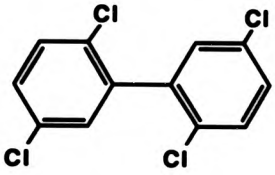

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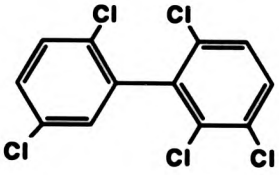

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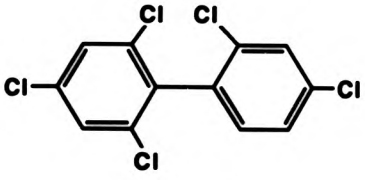

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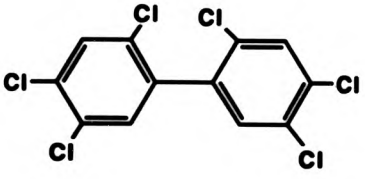

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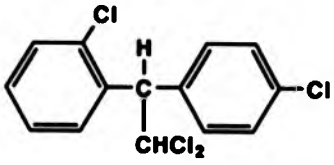

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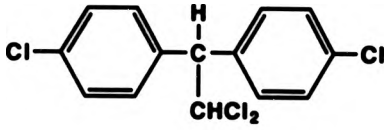

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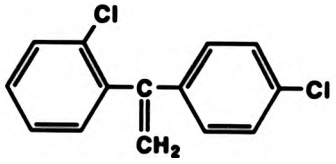

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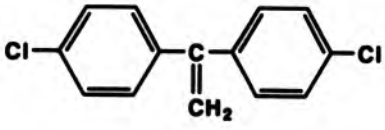

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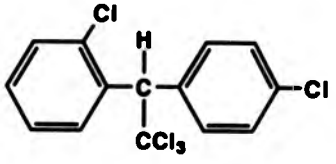

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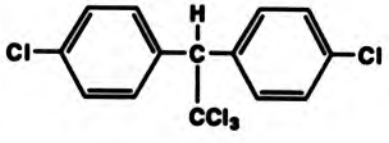

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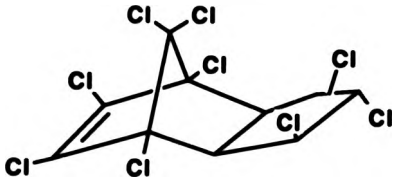

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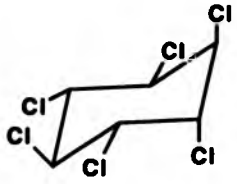
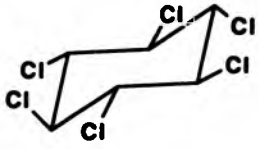


α HCH



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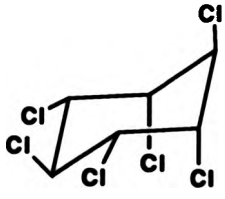

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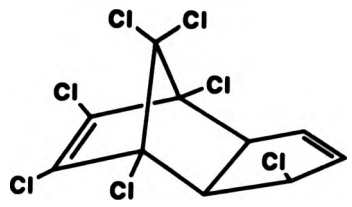

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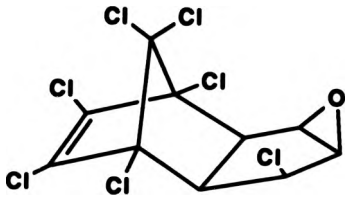

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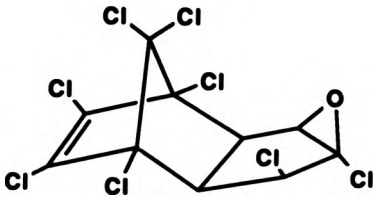
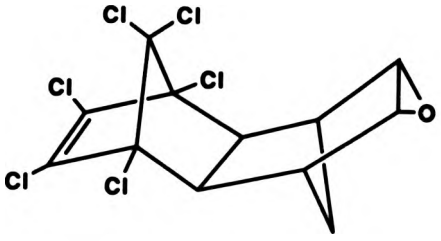


Oxychlordane



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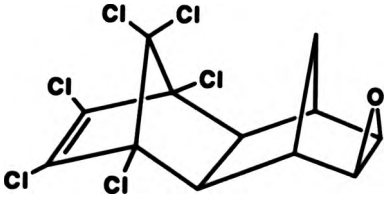

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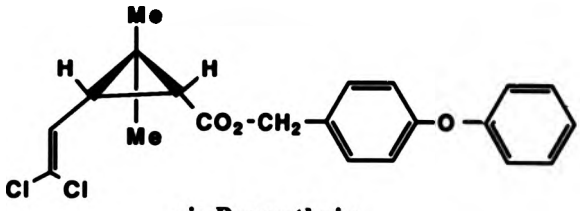

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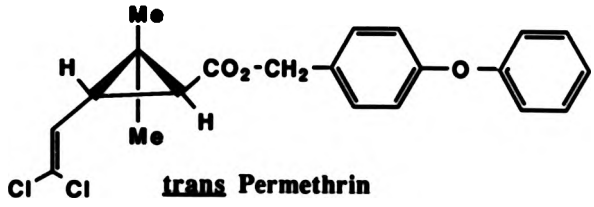

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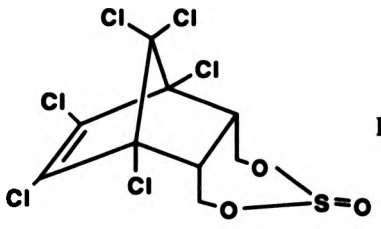

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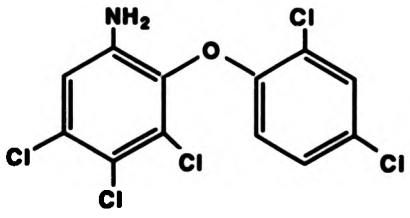

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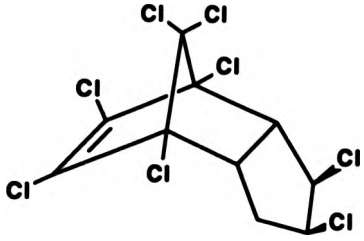
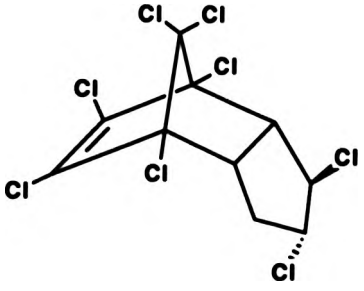
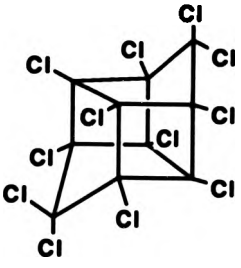


α Chlordane



γ Chlordane



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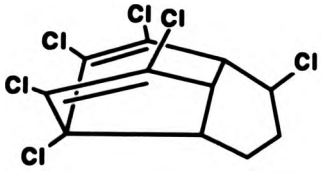
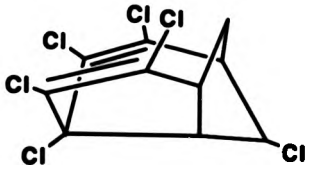


α Chlordene



γ Chlordene

APPENDIX 2

APPENDIX 2

The retention times and retention indices of the DCBEs and n-alkanes for the CPSil5-CB and CPSil8-CB columns using different starting temperatures and temperature programme rates are shown in Tables A1-A8. The starting temperatures used are as stated below:

A) Starting temperature 80°C with an isothermal period of one minute followed by the temperature gradient to an oven temperature of 260°C after which a temperature programme of thirty degrees per minute to a final temperature of 290°C was effected.

B) As for A except the starting temperature used was 100°C.

C) As for B, with a starting temperature of 120°C.

* Peak not recognised by acquisition programme.

Table A1 Retention times of DCBEs and n-alkanes on a CPSil5-CB column using different starting temperatures and a temperature programme rate of 3 deg min^{-1}.

	Conditions						
		A			B		
		Time (sec)			Time (sec)		
C11	173.6	179.2	172.6	119.5	118.3	113.8	118.1
C12	264.3	268.8	263.0	161.2	159.8	155.3	159.6
C13	394.5	399.1	394.0	229.4	227.4	223.5	227.1
D2	496.2	500.6	496.0	293.6	292.6	288.7	292.1
C14	563.9	568.2	563.9	329.7	328.8	325.2	328.5
D3	669.0	673.1	669.3	406.6	406.1	402.8	405.7
D4	869.9	874.21	870.6	556.2	556.3	553.3	556.1
C16	960.8	964.0	961.6	624.4	624.6	622.4	624.3
D5	1076.9	1081.3	1077.9	729.0	729.4	726.6	729.3
D6	1283.2	1287.6	1284.2	913.9	914.5	912.1	914.3
C18	1367.1	1370.3	1368.4	988.6	989.2	987.6	988.4
D7	1485.9	1490.5	1487.3	1104.5	1105.4	1103.1	1105.5
D8	1682.1	1686.2	1683.0	1293.5	1294.7	1287.7	1294.2
C20	1750.0	1754.3	1752.0	1359.1	1360.0	1358.7	1359.1
D9	1871.7	1876.6	1873.0	1479.9	1480.8	1479.8	1480.3
D10	2054.0	2058.9	2055.3	1660.2	1660.7	1659.2	1661.0
C22	2105.2	2109.6	2107.0	1710.2	1711.1	1709.6	1710.2
D11	2230.6	2235.7	2232.2	1835.2	1836.5	1834.8	1836.0
D12	2399.9	2405.7	2401.9	2004.1	2005.7	2004.0	2005.1
C24	2436.4	2437.9	2436.3	2038.1	2039.1	2038.2	2038.1
D13	2563.8	2568.9	2566.1	2167.9	2169.2	2167.7	2168.9
D14		2727.0		2325.7	2326.9		2326.8
C26	2721.9	2736.3	2724.0	2343	2343.1	2325.5	2343.0
D15	2874.2	2878.5	2876.0	2477.7	2479	2477.8	2478.0
C28							
D16	3021.1	3026.6	3023.7	2625.6	2626.8	2626.1	2626.9
C30	3291.3	3295.1		2895.5	2895.8	2895.5	2895.1

Table A1 (contd.)

	C									
C13	140.5	139.8	139.3	139.7	139.6	140.0	139.7	139.4	139.5	139.9
D2	174.8	173.8	173.3	174.0	173.6	174.1	173.9	173.4	173.5	174.0
C14	188.9	188.0	187.5	188.0	187.7	188.1	187.7	187.5	187.5	188.3
D3	234.3	233.0	232.5	233.3	232.6	233.2	232.6	232.2	232.4	233.4
D4	323.1	321.5	321.0	321.0	321.2	321.7	320.6	319.8	320.6	321.5
C16	361.7	360.0	359.4	359.2	359.4	360.1	358.6	358.0	358.9	359.9
D5	440.2	438.2	437.6	437.3	437.9	438.4	436.4	435.7	436.9	437.9
D6	580.9	578.5	577.8	577.1	578.2	579.1	576.1	575.4	577.0	578.1
C18	637.2	634.6	634.1	633.3	634.7	635.5	632.1	631.2	633.6	634.2
D7	740.8	738.1	737.5	736.7	738.5	739.0	735.3	734.7	737.0	737.7
D8	909.8	907.0	906.5	905.5	907.4	908.0	903.8	902.7	905.6	906.5
C20	967.8	964.8	962.4	963.5	965.3	966.3	966.2	960.7	963.6	964.1
D9	1083.3	1080.6	1080.2	1078.9	1081.6	1082.4	1077.0	1076.5	1079.2	1080.2
D10	1254.3	1253.9	1253.1	1252.1	1254.3	1255.8	1249.9	1249.8	1252.1	1253.2
C22	1304.5	1301.1	1300.7	1299.8	1302.3	1303.3	1297.8	1296.8	1299.9	1300.8
D11	1427.8	1425.1	1424.5	1423.0	1425.9	1427.1	1421.4	1420.4	1422.9	1424.6
D12	1595.0	1592.3	1591.4	1589.4	1592.8	1594.3	1587.9	1589.0	1590.5	1590.8
C24	1629.8	1624.7	1624.3	1622.2	1625.7	1627.2	1620.7	1620.1	1623.2	1623.3
D13	1756.8	1756.5	1753.6	1751.1	1754.8	1756.4	1750.5	1749.4	1752.3	1752.6
D14	1913.8	1911.4	1910.6	1908.2	1912.6	1913.6	*	1906.7	1909.9	1910.3
C26	1931.0	1928.4	1927.7	1925.3	*	1930.4	1924.2	1923.5	1927.0	1926.8
D15	2064.9	2063.1	2062.7	2059.4	2064.4	2064.8	2058.7	2058.3	2061.2	2061.1
C28 D16	2212.1	2210.8	2210.4	2206.8	2212.1	2212.2	2207.0	2206.0	2208.7	2209.8
C30	2482.2	2480.8	2480.1	2476.5	2482	2482.3	2476	2475.9	2478.8	2478.3

Table A2 Retention times of n-alkanes and DCBEs on a CPSil5CB column using a temperature programme of 5 deg min^{-1} and different starting temperatures.

	A		G C Conditions B							C	
	80°C 5deg min^{-1}		100°C 5 deg min^{-1}							120°C 5 deg min^{-1}	
C11	172.6	172.0	116.4	116.7	116.9	116.2	116.1	116.0	116.4	116.9	
C12	246.1	245.3	154.5	154.9	155.1	154.3	154.7	154.0	153.7	156.9	111.4 111.7
C13	344.9	344.2	213.2	213.7	213.8	212.8	213.2	212.6	212.4	213.4	140.8 141.0
D2	419.0	418.4	266.0	266.5	266.5	265.7	266.1	265.5	265.1	266.5	172.9 173.1
C14	462.8	462.8	293.2	293.7	293.8	292.8	293.1	292.5	292.2	293.9	185.4 185.6
D3	535.6	535.0	351.6	352.3	352.1	351.4	351.7	350.9	350.6	352.8	225.6 225.7
D4	665.6	664.8	458.1	458.5	458.4	457.8	457.9	457.0	456.9	459.9	298.2 298.1
C16	717.9	717.4	501.6	502.2	502.1	501.0	501.3	500.5	500.4	503.2	326.5 326.3
D5	795.7	795.4	574.0	574.5	574.4	572.7	573.1	573.9	573.0	576.1	386.5 386.3
D6	923.6	923.4	693.1	693.4	693.4	692.7	692.9	692.1	692.2	695.4	486.2 486.1
C18	969.8	969.7	736.5	736.7	736.4	735.7	735.8	735.1	735.2	738.3	521.2 521.3
D7	1048.8	1048.8	813.7	814.0	813.8	813.2	813.3	812.6	812.7	815.8	593.5 593.6
D8	1168.5	1168.5	930.9	931.2	930.9	930.1	930.2	929.7	929.7	933.2	702.8 702.8
C20	1204.5	1204.4	966.3	966.5	966.3	965.4	965.2	964.9	964.9	968.0	735.0 735.2
D9	1284.4	1284.6	1045.6	1045.6	1044.7	1044.8	1045.6	1044.2	1044.3	1044.5	812.5 813.0
D10	1395.5	1396.0	1156.1	1156.3	1156.0	1155.1	1155.4	1154.8	1154.7	1157.6	920.4 920.8
C22	1421.4	1421.6	1181.6	1181.7	1181.6	1180.2	1180.5	1180.0	1180.0	1182.8	946.9 945.6
D11	1503.2	1503.4	1263.1	1263.3	1263.2	1261.9	1262.3	1261.8	1261.6	1263.5	1025.9 1026.5
D12	1606.8	1607.3	1366.7	1366.8	1366.4	1365.3	1365.8	1365.2	1365.1	1369.8	1128.4 1129.1
C24	1621.5	1621.9	1381.5	1381.3	1381.2	1379.9	1380.3	1379.6	1379.6	1383.2	1142.8 1143.2
D13	1707.2	1707.0	1466.3	1466.1	1465.9	1464.2	1465.3	1464.5	1464.6	1464.4	1227.5 1227.9
D14	1801.9	1802.6	1562.0	1562.1	1561.7	1560.5	1561.2	1560.3	1560.3	1564.9	1324.6 1325.5
C26	1806.7	1807.2	1567.0	1566.8	1564.4	1565.2	1565.9	1564.8	1565.2		1327.6 1328.5
D15	1894.8	1895.1	1654.6	1654.7	1654.3	1654.3	1653.7	1652.7	1653.2	1657.5	
C28											
D16	1984.0	1984.7	1744.2	1744.0	1740.1	1742.5	1743.0	1742.2	1742.9	1747.3	1504.6 1505.1
C30			1902.8	1902.8	1902.4	1906.0	1901.5	1900.9	1901.0	1905.0	1663.0 1664.3

Table A3 Retention times of n-alkanes and DCBEs on a CPSil5CB column using a temperature programme of 7 deg min^{-1} and different starting temperatures.

G C Conditions

	Starting temp 80°C Programme Rate 7 deg min^{-1}			Starting temp 100°C 7 deg min^{-1}			Starting temp 120°C 7 deg min^{-1}	
C11	164.2	164.4	165.5	114.6	114.9	115.0		
C12	227.1	227.3	228.4	149.8	150.0	150.1	110.8	109.8
C13	307.9	307.5	308.8	201.2	201.6	201.6	138.2	137.3
D2	367.8	366.8	368.3	246.3	246.8	246.8	167.5	168.7
C14	399.6	398.5	400.1	269.6	268.0	268.0	178.3	179.3
D3	456.5	455.1	456.7	315.5	316.0	316.0	213.8	212.6
D4	553.3	551.7	553.4	398.5	399.1	399.1	271.4	273.7
C16	589.6	588.0	589.6	479.9	430.4	430.4	296.1	295.8
D5	649.6	649.5	649.3	486.6	486.8	486.8	345.3	345.2
D6	742.6	740.8	742.3	575.0	575.2	575.2	422.5	422.1
C18	773.6	771.7	773.2	604.3	604.7	604.7	447.3	446.8
D7	833.6	831.5	833.1	663.0	663.6	663.6	503.3	502.7
D8	920.2	918.0	918.8	768.4	748.7	748.7	584.5	583.7
C20	943.4	941.4	942.8	771.3	771.2	791.6	606.0	605.1
D9	1004.3	1001.9	1003.6	831.4	831.4	831.9	665.2	664.5
D10	1084.8	1082.4	1084.0	911.5	911.5	911.8	743.9	743.1
C22	1100.1	1098.1	1099.3	927.2	927.0	927.3	758.8	758.1
D11	1162.4	1160.2	1161.8	988.8	989.0	989.4	820.5	819.7
D12	1237.2	1234.7	1234.6	1063.4	1063.7	1063.8	894.4	893.6
C24	1246.6	1242.6	1244.4	1071.6	1071.7	1071.8	902.1	901.7
D13	1308.9	1306.5	1308.4	1135.2	1135.3	1135.6	965.8	965.4
D14 C26	1377 1378.6	1376.2	1377.6	1204.9	1205.6	1205.6	1035.3	1034.9
D15	1444.8	1442.5	1443.9	1271.7	1271.7	1271.7	1101.6	1101.2
C28	1504.2	1502.2	1503.5	1331.0	1331.2	1331.2	1161.2	1160.8
D16	1509.7	1507.7	1508.9	1336.0	1336.4	1336.6	1166.4	1166.1
C30	1616.9	1615.0	1619.9		1468.0	1468.3	1278.1	1277.8

Table A4 Retention times of n-alkanes and DCBEs on a CPSil8CB column using a temperature programme of 3 deg min^{-1} and different starting temperatures.

						A					
C12	341.2	339.7	339.7	339.4	337.5	334.0	336.1	336.8	336.0	336.6	336.5
C13	496.0	494.5	494.4	493.9	491.8	491.8	490.1	491.6	490.6	491.2	490.4
D2	649.5	647.8	647.4	646.8	644.8	645.6	643.8	645.1	644.0	645.0	644.3
C14	685.3	683.5	683.4	682.6	680.6	681.7	679.8	681.1	680.1	681.1	680.3
D3	840.6	838.8	838.4	836.7	835.6	837.2	835.3	836.7	835.8	836.7	836.4
D4	1054.4	1052.1	1052.6	1049.5	1051.6	1049.9	1051	1050.3	1051.4	1051.4	1051.6
C16	1106.1	1102.1	1102.8	1099.1	1099.7	1101.9	1100.1	1101.2	1100.5	1101.8	1102.1
D5	1271.2	1270.3	1269.6	1265.5	1266.3	1268.6	1267.1	1268.0	1267.4	1269.1	1269.1
D6	1483.4	1481.8	1481.2	1478.4	1478.2	1480.7	1479.1	1479.9	1479.2	1481.7	1481.7
C18	1523.8	1522.0	1521.4	1518.9	1518.6	1521.1	1519.5	1520.2	1519.3	1522.1	1522.2
D7	1692.8	1690.5	1689.5	1687.2	1687.2	1688.8	1687.4	1688.1	1687.0	1690.0	1690.3
D8	1892.4	1889.6	1886.1	1886.8	1886.9	1886.4	1886.9	1887.3	1885.8	1889.4	1889.7
C20	1917.6	1915.1	1913.2	1912.0	1912.0	1913.0	1911.6	1912.4	1911.0	1914.7	1914.9
D9	2086.0	2083.1	2081.1	2080.4	2080.4	2081.4	2079.9	2080.6	2079.0	2083.0	2083.1
D10	2271.8	2268.5	2265.9	2266.0	2266.1	2266.8	2265.2	2266.2	2264.0	2268.5	2268.4
C22	2280.6	2277.5	2274.6	2275.1	2275.1	2275.9	2274.2	2275.1	2273.2	2277.5	2277.5
D11	2450.7	2444.0	2444.7	2444.8	2445.2	2446.0	2444.1	2445.0	2443.2	2449.7	2447.8
C24	2616.2	2612.7	2609.5	2610.8	2611.0	2611.4	2610.0	2610.8	2609.1	2613.9	2613.5
D12	2623.2	2619.0	2617.0	2617.6	2618.1	2618.6	2617.2	2618.0	2616.0	2620.9	2620.7
D13	2789.0	2785.2	2781.6	2783.1	2783.3	2783.8	2782.8	2783.3	2781.5	2786.2	2786.4
C26	2926.4	2923.1	2919.5	2920.1	2921.2	2922.0	2920.8	2921.4	2919.5	2924.3	2924.3
D14	2949.1	2945.5	2941.5	2943.2	2943.1	2944.3	2942.9	2943.5	2941.8	2946.7	2946.5
D15	3103.0	3099.3	3095.0	3097.1	3097.3	3098.1	3096.8	3097.8	3095.5	3100.5	3100.6
C28	3217.9	3213.7	3209.3	3211.8	3212.3	3213.5	3212.0	3212.7	3210.7	3215.7	3215.8
D16	3252.8	3249.5	3243.9	3246.8	3247.0	3248.5	3246.6	3247.5	3245.5	3250.7	3250.7
C30	3489.1	3484.3	3479.5	3482.7	3483.5	3484.4	3482.9	3483.9	3481.6	3486.7	3486.8

Table A4 (contd.)

	B			C	
C12	205.7	205.6	207.3		
C13	292.7	292.6	294.3	186.7	187.3
D2	398.6	398.4	400.0	252.8	253.5
C14	416.6	416.4	418.0		
D3	538.3	537.9	539.9	331.8	332.4
D4	713.6	713.2	715.2	449.9	450.6
C16	751.7	751.4	753.4	470.8	471.6
D5	906.0	905.5	907.7	595.7	596.5
D6	1103.8	1103.2	1105.8	760.6	761.5
C18	1139.4	1138.7	1141.5	786.5	787.5
D7	1303.7	1303.0	1305.9	938.7	939.7
D8	1498.5	1497.2	1500.8	1120.4	1121.4
C20	1522.5	1521.0	1524.4	1140.3	1141.2
D9	1689.4	1687.9	1691.7	1303.5	1304.4
D10	1873.6	1871.9	1876.2	1483.3	1483.9
C22	1882.2	1880.5	1884.6	1490.5	1491.0
D11	2052.0	2050.4	2054.6	1659.2	1659.3
C24	2217.3	2215.9	2219.5	1822.6	1822.0
D12	2224.6	2223.1	2227.1	1830.3	1829.8
D13	2390.1	2389.1	2392.5	1994.9	1994.5
C26	2527.8	2527.2	2530.5	2132.1	2131.6
D14	2550.1	2549.7	2552.9	2154.7	2154.4
D15	2704.2	2704.1	2707.1	2308.7	2308.3
C28	2819.1	2819.2	2821.9	2423.6	2423.5
D16	2854.1	2854.7	2856.8	2458.4	2458.3
C30	3090.6	3091.3	3093.1	2694.4	2694.9

Table A5 Retention times of n-alkanes and DCEEs on a CPSil8CB column using a temperature programme of 5 deg min^{-1} and different starting temperatures.

	A			B				C				
C13								180.6	180.4	180.9	180.8	180.7
D2	237.5	237.9	237.8	237.5	236.8	237.4	238.3	237.1	236.8	237.5	237.2	237.1
C14												
D3	302.6	303.0	303.6	302.7	302.7	302.4	303.4	302.0	301.7	302.4	302.3	302.2
D4	392.1	392.7	393.9	392.3	393.0	392.1	393.0	391.6	391.2	392.1	392.0	391.9
C16	404.9	405.4	406.6	405.1	405.6	404.8	405.7	404.3	404.0	404.8	404.7	404.6
D5	495.8	496.3	497.7	495.5	496.9	495.5	496.3	494.9	494.5	495.3	495.4	495.2
D6	607.2	607.6	609.3	606.2	608.6	606.8	607.5	606.2	605.6	606.5	606.5	606.7
C18	619.8	620.4	622.0	619.2	621.4	619.6	620.3	619.0	618.5	619.2	619.3	619.3
D7	722.4	723.0	724.8	721.4	724.5	722.4	722.8	721.3	720.6	721.7	721.5	721.7
D8	837.6	838.1	840.1	836.1	839.3	837.9	837.8	836.1	835.3	836.6	836.2	836.3
C20	844.6	845.2	847.2	843.3	846.9	844.5	845.0	843.2	842.6	843.8	843.4	843.4
D9	951.3	952.0	954.5	949.9	954.1	951.5	951.6	950.7	949.2	950.7	949.9	950.0
D10 C22	1061.1	1062.4	1065.2	1060.3	1064.7	1062.2	1062.0	1061.0	1059.8	1061.0	1060.4	1060.3
D11	1169.7	1170.6	1173.7	1168.5	1173.4	1170.9	1170.4	1169.5	1168.2	1169.3	1168.6	1168
C24	1263.5	1264.4	1267.3	1262.4	1267.1	1264.8	1264.2	1262.1	1262.0	1263.0	1262.3	1262.5
D12	1273.9	1274.9	1278.0	1272.9	1277.5	1275.2	1274.7	1272.6	1272.6	1273.5	1272.8	1272.8
D13	1377.4	1375.3	1376.8	1373.4	1378.4	1375.5	1373.1	1373.1	1374.2	1373.2	1373.2	1373.8
C26	1452.4	1453.1	1456.6	1451.3	1456.2	1453.7	1453.2	1451.1	1451.3	1451.9	1451.9	1451.5
D14	1472.2	1472.6	1476.3	1471.0	1475.9	1473.6	1472.9	1470.6	1470.8	1471.6	1470.8	1471.1
D15	1565.6	1566.3	1569.7	1564.5	1569.6	1567.2	1566.5	1564.1	1564.6	1565.3	1565.6	1564.7
C28	1629.5	1630.0	1633.7	1628.9	1633.4	1631.1	1630.5	1628.3	1628.6	1629.2	1628.4	1628.7
D16	1650.7	1657.2	1661.2	1655.9	1660.9	1658.5	1657.8	1655.4	1655.7	1656.4	1655.5	1655.7
C30	1794.7	1795.0	1799.1	1793.9	1798.9	1796.1	1795.9	1793.4	1793.9	1794.3	1793.6	1794.0

Table A6 Retention times of n-alkanes and DCBEs on a CPSil8CB column using a temperature programme of 7 deg min^{-1} and different starting temperatures.

	A		B								C	
C12	277.6	276.3	188.5	188.0	188.2	188.6	187.8	187.9	188.3	187.4	140.4	139.2
C13	367.6	365.7	249.5	249.0	249.2	249.5	248.7	248.8	249.3	248.5	175.5	174.3
D2	452.0	450.0	319.2	318.8	318.9	317.2	316.3	316.4	317.1	316.8	224.4	223.1
C14	465.2	463.1	324.9	324.4	324.7	324.9	324	324.1	324.8	324.4		
D3	564.4	564.1	396.3	395.8	395.9	396.2	395.3	395.4	395.9	396.6	279.9	278.1
D4	647.3	644.9	486.9	486.5	486.5	485.9	485.9	485.9	486.5	487.2	351.6	349.8
C16	663.8	661.3	500.5	500.0	500.1	500.3	499.4	499.5	500.1	500.8	360.2	358.5
D5	746.3	743.8	580.0	579.6	579.5	579.8	579.0	579.1	579.7	580.5	431.9	429.9
D6	841.5	839.1	671.9	671.6	671.5	671.9	670.8	670.9	671.6	672.5	515.8	513.7
C18	852.1	849.3	681.7	681.3	681.2	681.6	680.7	680.6	681.3	682.3	523.1	520.9
D7	934.0	931.6	762.6	762.2	762.0	762.5	761.5	761.6	762.5	763.2	601.2	599.2
D8	1020.6	1019.8	849.9	849.4	849.3	849.6	848.8	848.8	849.6	850.4	688.4	683.9
C20												
D9	1107.6	1105.3	934.8	934.4	934.1	934.5	933.6	933.6	934.5	935.4	768.1	766.8
D10	1189.3	1187.2	1016.1	1015.9	1015.6	1015.9	1016.7	1016.5	1015.8	1016.2	848.2	847.1
C22												
D11	1268.1	1265.9	1094.7	1094.3	1094.0	1094.4	1093.7	1093.6	1095.3	1094.2	925.2	925.2
C24	1333.3	1331.2	1159.9	1159.5	1159.3	1159.8	1158.8	1158.9	1159.7	1160.3	990.4	989.8
D12	1343.9	1341.8	1170.5	1170.0	1169.6	1170.3	1169.3	1169.3	1170.2	1170.8	1001.1	1000.5
D13	1416.9	1414.7	1243.1	1242.5	1242.4	1242.9	1242.0	1241.9	1242.8	1243.4	1073.3	1072.9
C26	1469.9	1468.2	1296.7	1296.2	1295.9	1296.4	1295.6	1295.6	1296.4	1297.0	1126.3	1126.3
D14	1487.0	1485.2	1313.7	1313.1	1312.9	1313.6	1312.5	1312.4	1313.5	1314.0	1143.5	1143.2
D15	1554.7	1552.9	1381.3	1380.7	1380.4	1381.2	1380.1	1380.0	1381.0	1381.5	1210.9	1210.9
C28	1597.8	1596.9	1424.4	1423.7	1424.5	1424.5	1423.4	1423.2	1423.3	1424.6	1254.1	1254.2
D16	1620.4	1618.7	1446.9	1446.6	1446.2	1446.8	1445.9	1445.9	1446.8	1447.2	1276.5	1276.7
C30	1717.0	1715.1	1543.6	1543.1	1543.0	1543.7	1542.5	1542.4	1543.5	1544.0	1373.4	1373.3

Table A7 Retention indices of DCBEs on a CPSil5CB column using different temperature programme and starting temperatures.

	Starting temp. 80°C Prog. rate 3 deg min^{-1}			Starting temp. 100°C Prog. rate 3 deg min^{-1}			
D2	1363	1362	1363	1367	1367	1369	1369
D3	1455	1455	1454	1462	1463	1459	1459
D4	1556	1556	1556	1561	1561	1559	1559
D5	1657	1657	1657	1659	1659	1659	1660
D6	1758	1759	1758	1760	1760	1760	1761
D7	1860	1861	1861	1862	1862	1862	1862
D8	1963	1963	1963	1964	1966	1964	1966
D9	2066	2067	2067	2067	2067	2067	2067
D10	2170	2170	2171	2171	2170	2171	2171
D11	2274	2275	2275	2275	2275	2274	2275
D12	2378	2380	2379	2379	2379	2379	2379
D13	2483	2484	2483	2483	2484	2483	2483
D14	2589	2589	2588	2588	2588	2589	2588
D15	2695	2694	2694	2693	2694	2694	2694
D16	2800	2800	2800	2800	2800	2800	2800

	Starting temp 120°C				Programme rate 3 deg min^{-1}					
D2	1373	1372	1373	1373	1373	1373	1372	1373	1373	1372
D3	1473	1473	1473	1474	1473	1473	1473	1473	1473	1473
D4	1569	1569	1569	1569	1569	1569	1569	1569	1569	1568
D5	1660	1660	1660	1661	1660	1661	1661	1660	1660	1661
D6	1762	1762	1762	1762	1762	1762	1762	1762	1762	1762
D7	1866	1866	1866	1866	1866	1866	1866	1866	1866	1866
D8	1966	1966	1966	1966	1966	1966	1966	1966	1966	1966
D9	2068	2068	2068	2068	2068	2068	2068	2068	2068	2068
D10	2171	2171	2171	2171	2171	2171	2171	2171	2171	2171
D11	2275	2275	2275	2275	2275	2275	2275	2275	2275	2275
D12	2379	2379	2379	2379	2379	2379	2379	2379	2379	2379
D13	2483	2484	2483	2483	2483	2483	2483	2483	2483	2484
D14	2588	2588	2588	2588	2588	2589	2589	2589	2588	2589
D15	2694	2694	2694	2694	2694	2694	2694	2694	2694	2693
D16	2800	2800	2800	2800	2800	2800	2800	2800	2800	2800

Table A7 (contd.)

	G C Conditions					
	st. temp. 80°C Prog.rate 5 deg min^{-1}		st. temp. 100°C prog.rate 5 deg min^{-1}		st. temp. 120°C prog. rate 5 deg min^{-1}	
D2	1365	1365	1370	1370	1373	1373
D3	1458	1458	1461	1461	1472	1472
D4	1559	1559	1561	1561	1568	1569
D5	1661	1661	1662	1663	1664	1664
D6	1763	1763	1764	1764	1765	1766
D7	1865	1865	1866	1866	1868	1868
D8	1968	1968	1968	1969	1970	1969
D9	2072	2072	2072	2072	2073	2073
D10	2175	2176	2176	2175	2177	2177
D11	2280	2280	2280	2279	2280	2281
D12	2384	2385	2384	2385	2385	2384
D13	2490	2490	2489	2490	2490	2491
D14	2594	2594	2594	2595	2594	2595
D15	2698	2698	2699	2699	2699	2697
D16	2800	2800	2803	2800	2805	2800

Table A7 (contd.)

	G C Conditions						
	st. temp 80°C		st. temp. 100°C			st. temp 120°C	
	prog. rate 7 deg min^{-1}		prog. rate 7 deg min^{-1}			prog. rate 7 deg min^{-1}	
D2	1366	1366	1371	1371	1370	1376	1374
D3	1460	1460	1464	1463	1462	1474	1472
D4	1562	1562	1563	1564	1564	1570	1569
D5	1664	1663	1664	1665	1665	1666	1668
D6	1765	1765	1766	1765	1767	1769	1769
D7	1869	1869	1869	1869	1869	1872	1871
D8	1972	1972	1972	1972	1972	1973	1972
D9	2076	2074	2075	2075	2075	2077	2076
D10	2179	2179	2179	2179	2179	2181	2180
D11	2284	2284	2283	2284	2284	2284	2283
D12	2390	2388	2388	2388	2388	2388	2386
D13	2494	2494	2495	2494	2494	2494	2495
D14	2600	2600	2600	2600	2600	2600	2600
D15	2702	2702	2704	2703	2703	2703	2705
D16	2802	2802	2804	2808	2805	2808	2810

Table A8 Retention indices of DCBEs on a CPSil8CB column using different temperature programme of 3 deg min^{-1} and different starting temperatures.

	Starting temp 80°C				Programme rate 3 deg min^{-1}						
D2	1382	1382	1382	1382	1382	1382	1382	1382	1382	1382	1382
D3	1475	1475	1475	1475	1475	1475	1475	1475	1475	1475	1475
D4	1577	1577	1576	157	1576	1576	1576	1576	1576	1576	1576
D5	1679	1679	1679	1679	1679	1679	1679	1679	1679	1679	1679
D6	1780	1780	1780	1780	1780	1780	1780	1780	1780	1780	1780
D7	1884	1884	1884	1884	1884	1884	1884	1884	1884	1884	1884
D8	1987	1987	1987	1987	1987	1987	1987	1987	1987	1987	1987
D9	2091	2091	2091	2091	2091	2091	2091	2091	2091	2091	2091
D10	2195	2195	2195	2195	2195	2195	2195	2195	2195	2195	2195
D11	2299	2999	2300	2299	2299	2299	2299	2299	2299	2299	2299
D12	2404	2404	2404	2404	2404	2404	2404	2404	2404	2404	2404
D13	2510	2509	2509	2509	2509	2509	2509	2509	2509	2509	2510
D14	2615	2615	2615	2615	2615	2615	2615	2615	2615	2615	2615
D15	2719	2719	2719	2719	2719	2719	2719	2719	2719	2719	2719
D16	2825	2825	2825	2825	2825	2825	2825	2825	2825	2825	2825

	St. temp. 100°C Prog. rate 3 deg min^{-1}			St. temp 120°C Prog rate 3 deg min^{-1}	
D2	1388	1388	1388	1400	1400
D3	1479	1479	1479	1485	1485
D4	1579	1579	1579	1585	1584
D5	1681	1681	1681	1685	1685
D6	1782	1782	1782	1785	1785
D7	1885	1885	1885	1887	1887
D8	1987	1987	1987	1989	1989
D9	2091	2091	2091	2092	2092
D10	2195	2195	2195	2196	2196
D11	2299	2299	2299	2300	2300
D12	2405	2405	2405	2405	2405
D13	2510	2510	2510	2510	2510
D14	2615	2615	2615	2615	2615
D15	2719	2719	2719	2719	2719
D16	2825	2825	2825	2825	2825

Table A8 (contd.)

Programme Rate 5 deg min^{-1}

	Starting Temperature 80°C			Starting Temperature 100°C				Starting Temperature 120°C				
D2	1400	1400	1400	1400	1400	1400	1400	1400	1400	1400	1400	1400
D3	1487	1487	1487	1487	1487	1487	1487	1487	1487	1487	1487	1487
D4	1587	1587	1587	1587	1587	1587	1587	1587	1587	1587	1587	1587
D5	1688	1688	1688	1688	1688	1688	1688	1688	1688	1688	1688	1688
D6	1789	1789	1789	1789	1789	1789	1789	1789	1789	1789	1789	1789
D7	1891	1891	1891	1891	1891	1891	1891	1891	1891	1891	1891	1891
D8	1994	1994	1994	1994	1994	1994	1994	1994	1994	1994	1994	1994
D9	2097	2097	2097	2097	2097	2097	2097	2097	2097	2097	2097	2097
D10	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200
D11	2305	2305	2306	2305	2306	2305	2305	2305	2305	2305	2305	2305
D12	2411	2411	2411	2411	2411	2411	2411	2411	2411	2411	2411	2411
D13	2516	2516	2516	2516	2516	2516	2516	2516	2516	2516	2516	2516
D14	2622	2621	2622	2622	2622	2622	2622	2622	2621	2621	2621	2621
D15	2726	2726	2726	2726	2726	2726	2726	2726	2725	2726	2726	2726
D16	2832	2832	2832	2832	2832	2832	2832	2832	2832	2832	2832	2832

Table A8 (contd.)

	G C Conditions										
	St. temp. 80°C Rate 7 deg min^{-1}		St. temp. 100°C Rate 7 deg min^{-12}							St. temp. 120°C Rate 7 deg min^{-}	
D2	1391	1391	1391	1391	1390	1391	1391	1391	1391	1400	1400
D3	1481	1481	1484	1484	1484	1484	1484	1484	1484	1489	1489
D4	1583	1583	1585	1585	1585	1585	1585	1585	1585	1589	1589
D5	1685	1686	1686	1686	1686	1687	1687	1688	1688	1690	1690
D6	1788	1788	1789	1789	1789	1789	1789	1789	1789	1791	1791
D7	1895	1893	1896	1896	1896	1896	1893	1896	1894	1896	1896
D8	2000	1997	2000	2000	2000	2000	2000	2000	2000	2000	2000
D9	2100	2100	2100	2100	2100	2100	2100	2100	2100	2100	2099
D10	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200
D11	2307	2307	2307	2307	2307	2307	2307	2307	2307	2307	2307
D12	2415	2415	2415	2415	2415	2415	2415	2415	2415	2416	2415
D13	2521	2521	2521	2520	2521	2521	2520	2520	2520	2521	2521
D14	2626	2626	2626	2626	2626	2626	2626	2626	2626	2626	2626
D15	2731	2731	2731	2730	2730	2730	2730	2730	2730	2731	2730
D16	2837	2837	2837	2837	2836	2836	2836	2837	2837	2837	2837

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