APPLICATION OF SOLID PHASE MICROEXTRACTION TO THE EXTRACTION AND ANALYSIS OF ORGANIC COMPOUNDS

by

Frank Quinn

A thesis submitted for the degree of
Master of Science

Declaration

I hereby certify that this material, which is submitted for assessment on the programme of study leading to the award of Master of Science is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of this work.

Signed: Frank Quinn Date: 26/09/01

Frank Quinn

ACKNOWEDGEMENTS

I wish to thank my supervisor at Dublin City University, Professor Malcolm Smyth for his guidance, encouragement and advice.

I would like to say a special word of thanks to my supervisor at Enterprise Ireland, Mr. Kevin Dynes, for all his help throughout the course of my time in the trace organics laboratory. I would like to express my gratitude to Jean Smith, John Nugent, Edel Stanley and Brian Brennan of the laboratory for making me part of their team.

I would also like to thank Dr. Frances Weldon for her help with the preparation of this thesis.

Lastly I would like to say a very big thanks to my parent's for their constant support and encouragement.

CONTENTS

CHAPTER 1.0
Introduction

CHAPTER 2.0
SPME and its application to the analysis of Volatile Organic Compounds

CHAPTER 3.0
Plasticisers in Children's Toys

CHAPTER 4.0
Determination of Leaching Rates of DINP from Children's Toys and Childcare Articles using SPME/LLE-GC-MS

APPLICATION OF SOLID PHASE MICROEXTRACTION TO THE EXTRACTION AND ANALYSIS OF ORGANIC COMPOUNDS

Abstract

Solid Phase Microextraction (SPME) is a sample preparation and introduction technique in which analytes are extracted into a small volume of extracting phase coated on a fused silica fibre and subsequently desorbed within the analytical instrument. This technique was investigated for the extraction of Volatile Organic Compounds (VOCs) from aqueous matrices and for the determination of phthalate ester leaching from plastic items.

Using a 100μm PDMS fibre and GC-MS analysis, VOCs could be determined in water samples at the low parts per billion level. This was found to match Purge and Trap-GC-MS detection limits for most VOCs but with the additional advantage of easier operation, shorter analysis times and potential on-site sampling.

PVC toys and childcare articles were screened for leaching of the phthalate ester Diisononyl phthalate (DINP) by shaking a sample disc in a saliva simulant followed by extraction of the leached phthalates. An 85μm polyacrylate fibre was used to extract the leached phthalates and analysis was carried out using GC-MS. Solvent extraction was carried out for comparative purposes. Articles leaching phthalates above xμg / 10cm^2 / min could be identified.

Chapter One

Literature Review

Solid Phase Microextraction – A Review of Principles and Applications

1.1 INTRODUCTION

Current requirements for the determination of organic species require the analyte to be determined at trace levels (ng/l, μg/l and mg/l) in matrices that are quite often of a very complex nature. Quantitation of Volatile Organic Compounds (VOCs) in industrial wastewater, screening of blood for drugs of abuse, and residual determination of pesticides in groundwater are examples of such analyses. The demand for sensitive methods which are quick, cost effective, and can deal with large sample numbers, is ever increasing.

Gas Chromatography (GC) is widely used in trace organic analyses. Developments in column technology have resulted in the availability of high-resolution capillary columns, enabling the analytes of interest to be separated from each other and from other components in the sample. The large advances in electronics that have occurred over the last few decades have benefited this area also. Gas chromatographic detectors have become very sophisticated and capable of acquiring large amounts of data. The flame ionization detector (FID) has emerged as the most widely used and generally applicable detector for GC, with a range of other detectors such as the thermal conductivity detector, the thermionic detector, the electron capture detector and the flame photometric detector available for specific applications. Due to its sensitivity, and its ability to enable positive analyte identification mass spectrometeric (MS) detection has many advantages to offer over these other detectors although ultimately the detector of choice depends on the application.

Although improvements in technology have resulted in automated methods being developed with high sample throughput, many analyses still require a lengthy sample preparation or sample pre-concentration step which can very often be the longest step in the analysis. These sample preparation steps include closed-loop stripping, solid-phase extraction (SPE), liquid-liquid extraction (LLE), purge and trap (P+T) and headspace techniques. Although these techniques are time-consuming, they are necessary in order to prevent carryover problems (sample clean-up) and to offset limitations in detection (sample pre-concentration).

1.2 SAMPLE PREPARATION TECHNIQUES

In the early 1990s, a new technique which incorporated analyte extraction, pre-concentration and introduction in one easy step was introduced by Arthur and Pawliszyn at the University of Waterloo in Canada[1]. This technique, known as Solid-phase microextraction (SPME) was developed by taking a fused silica fibre of 1 cm in length and coating it with a suitable adsorbent material. The resulting SPME fibre was then exposed either directly to the sample or to the headspace above the sample permitting partitioning of the analytes into the coating. The now "loaded" fibre was then transferred to the heated injection port of a GC where the analytes were thermally desorbed, swept onto the analytical column, separated and detected. **Figure 1.1** shows the current commercially available manual SPME fibre holder.

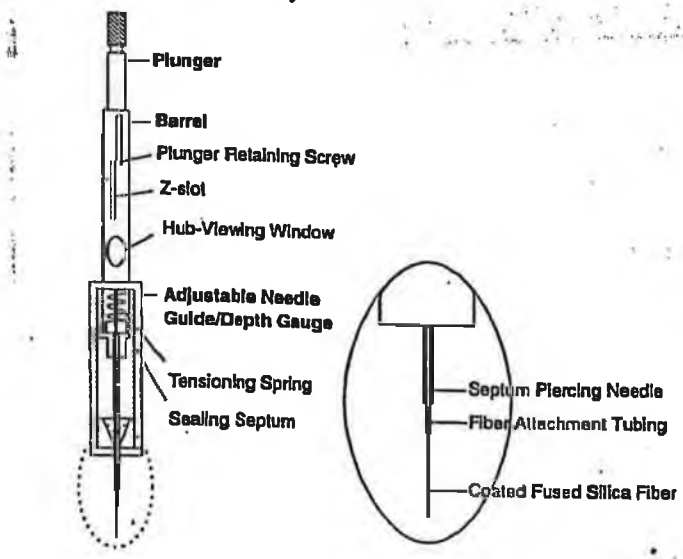

Figure 1.1: Design of the commercially available SPME device[2]

The fused silica fibre is connected to stainless steel tubing which is used to increase the mechanical strength of the fibre assembly for repeated sampling. The stainless steel tubing is then contained in a specially designed syringe needle. During SPME the needle is used to puncture vial and injector septa. The plunger is depressed once inside the vial or injector thereby exposing the fibre.

Many well-established classical sample preparation techniques have successfully been incorporated in several validated methods. SPME is a relatively new technique with the potential to replace techniques such as LLE, SPE, P+T, headspace and closed-loop stripping in the future, as it does not suffer from many of the disadvantages these other methods do.

LLE uses large quantities of expensive solvents. In today's environmentally conscious society, SPME is an attractive alternative because it does not use any solvents. The use of solvents also has implications from a health and safety point of view. Exposure of laboratory personnel to solvents on a daily basis can have an adverse effect on health. Most organic solvents are also highly flammable and therefore constitute a fire hazard in the laboratory. Formation of emulsions during liquid-liquid extractions of solutions containing proteins may also be problematic.

SPE of dirty samples can result in plugging of the SPE cartridge. This problem does not arise with SPME because the fibre is simply immersed in the sample or exposed to the headspace above the sample. In cases of extremely dirty samples, the fibre can be surrounded by a semi-permeable membrane to prevent damage to the fibre[3]. SPME requires a minimum amount of sample (1-2 ml) compared to other techniques which require much larger volumes, e.g. SPE can require between one to two litres of sample to achieve similar detection limits for certain semi-volatiles[4].

Automation of P+T and SPE often requires bulky expensive instrumentation which may be more expensive to run and maintain, and technically more complex than the simple, adapted, direct liquid injection auto-sampler required for automated SPME. In contrast, the time-consuming method of closed-loop stripping and LLE does not lend itself to automation.

Sample carryover may be problematic with some methods. Although this is also true of SPME, it is more easily remedied. Increasing the desorption temperature and / or the desorption time can prevent analyte carryover on the SPME fibre. By comparison, SPE, LLE and P+T carryover demands that the instrument lines and sample containers are thoroughly washed with the expensive extracting solvent or purge gas.

Optimisation of parameters for SPME extraction is essential. These parameters will be described in detail later but each SPME analysis can basically be described as follows

1. SPME has in the majority of cases been applied to the analysis of organic compounds, so therefore the sample is collected in a glass vessel which has been thoroughly washed, silanised and the vessel then sealed with a PTFE cap. Collection of the sample in this way prevents any losses of the analyte to the atmosphere or to the surfaces of the container.

2. Conditioning of the SPME fibre prior to analysis is an essential step. Blank analysis of a new fibre will often reveal a number of extraneous peaks – these residual interfering compounds can be due to the fibre coating itself or to monomers of the adhesive used to connect the fibre to the stainless steel tubing[5]. Conditioning involves baking the fibre in the injector at a designated temperature for a time period as described in the literature accompanying the fibres. This results in removal of these extraneous peaks. Some fibres require conditioning for long periods of time to remove residual compounds; however, with some fibre types certain peaks always remain.

3. The stainless steel needle, which acts as a protective case for the fibre, is used to puncture the PTFE cap and the plunger then depressed resulting in fibre exposure as shown in **Figure 1.2**.

4. The fibre remains exposed for a pre-determined time. Once this equilibration time has elapsed the fibre is retracted into the needle, which is removed from the sample container and used to puncture the septum of the GC's injector.

5. The plunger is again depressed, this time exposing the fibre in the heated injector liner where the analytes become thermally desorbed and swept on

to the GC column. Desorption time and temperature are pre-determined. Removal of the fibre from the injector completes the process and it is now ready for another analysis.

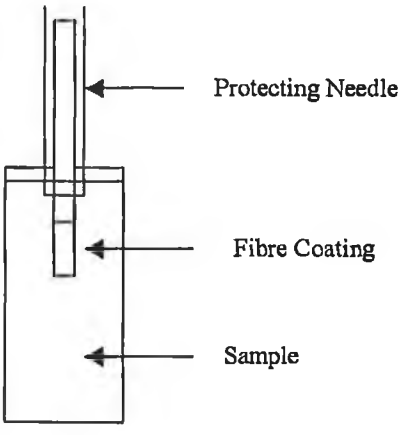

Figure 1.2: Figure showing a fibre extracting a sample.

1.3 APPLICATIONS OF SPME

Since the first publication on SPME appeared back in 1990 there have been many publications on this topic. Although a significant proportion of these have been dedicated to environmental pollutants, recently the range of reported applications has widened in scope, highlighting the growing popularity of this technique with analysts world-wide. The following brief review of applications in the literature illustrates the diversity and potential of SPME. Applications can be categorised as follows

- Water
- Air
- Medical
- Food
- Forensic
- Miscellaneous

1.3.1 Water: The analysis of water for organics, particularly VOCs, has been the area in which most SPME work has been done. Belardi and Pawliszyn published one of the first papers on this topic back in 1989 in which they described

how a chemically modified fused silica fibre could be used to extract organics from water and rapidly transfer the extract to a capillary column[6]. The following decade saw many papers outlining how SPME had been used in the analysis of BTEX (Benzene, Toluene, Ethylbenzene and Xylene), substituted benzenes and halogenated VOCs. Gorecki *et al.* described how a PDMS fibre could be used for the rapid analysis of complex organic[7]. Headspace sampling using a 15μm PDMS fibre enabled the separation of BTEX components in ca. 12 seconds using high speed gas chromatography. Nilsson *et al.* used SPME to determine concentrations of VOCs of varying volatility in drinking water[8]. Reasonable agreement between results obtained by SPME and Purge and Trap were obtained although detection difficulties were encountered for some of the very light VOCs.

With the commercial availability of new fibre coatings came publications on SPME and the semi-volatile organics, including Polynuclear Aromatic Hydrocarbons, Polychlorinated Biphenyls, Pesticides, Herbicides and Phenols. Yang *et al.* combined SPME with GC-ECD for the analysis of polychlorinated biphenyls in ocean, wetland and leachate water samples[9]. Concentrations in water samples obtained by 15-min SPME extractions compared favourably with those obtained by toluene extractions, demonstrating that SPME combined with GC is a useful technique for the rapid determination of PCBs in water samples. Magdic *et al.* found sub ng/l level analysis of eighteen organo-chlorine pesticides in river and lake water samples possible when using a 100μm PDMS fibre and GC with electron-capture detection[10]. PAHs have been analysed by Chen *et al.* using SPME-HPLC[11]. A 7μm PDMS fibre was used to extract PAHs from spiked water samples. The good reproducibility in both retention time and peak area demonstrates that SPME-HPLC can be used for both qualitative and quantitative analyses. Hageman *et al.* undertook coupled subcritical water extraction with solid phase microextraction for the determination of semi volatiles in environmental solids[12]. After a 15-60 minute subcritical water extraction, the water was analysed by SPME for PAHs. Recoveries ranging from 60 to 140% better than conventional solvent extraction were achieved. SPME has also been applied to the determination of the two most common causes of odours in water, geosmin and 2-methylisoborneol. McCallum *et al.* investigated the use of a number of different fibre chemistries in the determination of these two compounds and found their

determination possible at levels below their odour threshold[13]. Artificial musk fragrances are another class of compounds extracted from water using SPME. These compounds are surface water contaminants and are of interest due to their possible toxicology. Winkler *et al.* used a number of different fibre types but found PDMS-DVB fibres to give the best recoveries[14]. Typical detection limits for three polycyclic musk fragrances and one nitro musk fragrance were between 14 and 22ng/l.

1.3.2 Air: The current methods for analysis of organics in air include trapping of the analyte on a sorbent bed. Sampling can be either active or passive. Active sampling involves pumping the air sample through a tube packed with the sorbent. Passive sampling relies on diffusion of the analyte through the air and onto the sorbent. A number of sorbents in common use are Tenax (for compounds in the boiling range 100 – 300 °C), Chromosorb 106 (for compounds in the boiling range 50 – 200 °C) and graphitised carbons such as Carbograph, Carbotrap and Carbopack (for compounds in the boiling range 0-60 °C). After trapping of the analyte the tubes are thermally desorbed in a special unit called an automatic thermal desorber (ATD). Air sampling can also be carried out using grab sampling with stainless steel canisters or nylon bags. This latter sampling method requires the analyte to be present at relatively high concentrations.

SPME can also be applied to the analysis of air samples. The fibre is simply exposed in the area under investigation, capped and taken back to the laboratory for analysis. Using this approach Chai and Pawliszyn developed a simple and efficient method for extraction of VOCs in air[15]. Analytes were detected at the part per trillion to sub part per billion level and the precision of the method was determined to be 1.5 - 6 % RSD. More recently Koziel *et al.* demonstrated how SPME fibres could be used for sampling of VOCs from air[16]. The method claims to be the fastest extraction technique for air sampling of typical airborne VOC concentrations with sampling times of 5s to 1 min employed. Another approach to air quality analysis using SPME was undertaken by Guidotti *et al.*[17]. Phthalate esters and pesticides are organic air pollutants that collect in rainwater. Rainwater samples were collected and analysed by SPME-GC-MS and found to contain these organics. Determination of airborne amines[18] and total petroleum hydrocarbons[19] have also been carried out using SPME.

More diverse applications of SPME to air analysis include the study of airborne insect pheromones[20], monoterpenes from conifer needles[21] and static-air emissions of ammonia, methylamine and putrescine from a lure for the mexican fruit fly[22].

1.3.3 Clinical/Forensic: The analysis of biological samples for organics has always been challenging due to the very complex nature of the matrix. SPME is a suitable extraction method for analytes such as drugs of abuse and therapeutic drugs in urine and other biological matrices. Lord and Pawliszyn recently published a comprehensive review on the microextraction of drugs[23] while Ulrich[24] and Mills and Walker[25] reviewed the use of SPME in biomedical analysis. Other forensic applications of SPME include the analysis of fire debris for accelerants[26] and urine for ethanol[27].

1.3.4 Food: The analytical laboratory plays a large part in the overall quality control system in the food and drink industries. SPME has helped companies identify those compounds that are responsible for off-flavours and taints in their products. It has also enabled complete profiles of those compounds responsible for a product's unique taste and flavour to be obtained. In the drinks industry, for example, whiskey manufacturers use a variety of analytical techniques, including SPME, to profile their products[28]. This prevents the practice of blending superior brands with inferior brands without the prior consent of the manufacturer. Reports on the use of SPME in the characterisation of commercial vodkas[29], the analysis of wine bouquet components[30] and flavour volatiles in fruit beverages[31] are available.

1.4 DEVELOPMENT AND OPTIMSATION OF A SPME METHOD

Any analysis, which utilises SPME, has a number of parameters unique to the SPME process to optimise. Failure to pay proper attention to these variables can result in a method which may have high limits of detection and quantitation and poor reproducibility. In addition, severe carryover problems may be encountered. Below is a description of each of these parameters and a discussion on the effect of each on the analysis.

1.4.1 Extraction Time

There are two stages in the SPME process

(i) Partitioning of the analyte between the sample matrix and the fibre coating

(ii) Desorption of the analyte in the analytical instrument.

The first part is an equilibrium process - the amount of analyte partitioning into the coating at a particular concentration depending mainly on the extraction time. Chloroform can reach equilibrium with a PDMS coating in one minute[32] whereas some of the semi-volatile compounds take much longer, for example, o-cresol takes over 80 minutes to reach equilibrium with the polyacrylate fibre[33]. When developing a method it is preferable to keep the extraction time the same or less than the GC run-time as this means that extraction of one sample can be carried out simultaneously with analysis of another sample. In cases where the time taken for the SPME extracting phase to reach equilibrium with the sample exceeds the GC run-time an extraction time less than the equilibration time is generally decided upon. This is done in order to keep the analysis time to a minimum, that is, in cases where an automated run of a number of samples is being carried out the GC run time governs the overall automated run time. When using extraction times shorter than the equilibration time care should be taken to ensure that each extraction is carefully timed and that agitation conditions remain constant, otherwise the amount extracted will vary from run to run. Many of the other parameters discussed below have an important effect on the extraction time and must be considered before an extraction time is decided on.

1.4.2 Extraction Mode

There are three modes of SPME extraction as depicted in **Figure 1.3**

(i) Headspace Sampling: The SPME fibre is exposed to the headspace above the sample. This mode is ideal for the extraction of volatile compounds. It is important to ensure that the headspace volume remains the same for each extraction and time is given to allow the analyte partition between the sample and headspace as otherwise the repeatability will be poor.

(ii) Immersion Sampling: In this mode, which is used for less volatile compounds, the SPME fibre is in direct contact with the aqueous matrix. Whilst equilibration times are longer when using immersion sampling agitation of the sample can result in a significant reduction.

(iii) Membrane-Protected SPME: This involves surrounding the fibre with a membrane. The purpose of this is to protect the fibre in very dirty samples by allowing the analytes to diffuse into a "clean" area around the fibre. In this way compounds insufficient volatility for the headspace method can be determined in severely contaminated samples without any damage to the fibre.

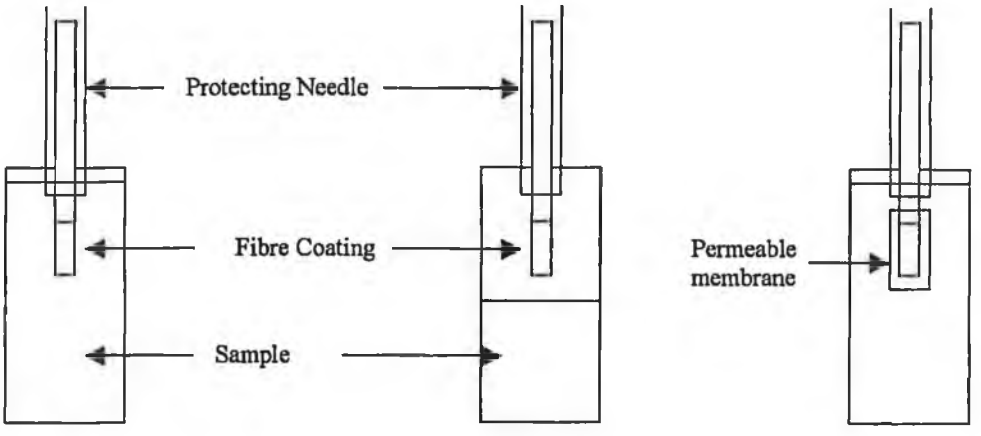

Figure 1.3: Modes of SPME operation: (a) direct extraction, (b) headspace SPME, (c) membrane-protected SPME.

When micro-extracting a sample agitating the sample can favourably enhance the extraction kinetics[34]. Agitation removes the depletion zone surrounding the fibre and therefore mass transfer of the analyte to the coating is greatly enhanced. The mode of agitation chosen is ultimately determined by the application.

(i) Static Sampling: SPME may be carried out under static conditions. The fibre is either immersed in the sample or placed in the headspace above the sample. No agitation of the sample takes place. Static headspace extraction is most commonly used for solid-phase microextraction of volatile organic compounds.

(ii) Magnetic Stirring: This is the most commonly used means of agitation for non-automated SPME. A stirring bar is placed in the sample vial and the vial placed on a magnetic stirrer. One disadvantage of this method is that the stirring bar can sometimes be a source of contamination. Analytes can become adsorbed on the bar resulting in sample carryover. Proper use of blanks will indicate this problem and precautions can be taken to prevent it.

(iii) Sonication: Sonication ensures very thorough mixing leading to the shortest extraction times. The main disadvantage of this approach is that it can heat the sample resulting in a decrease in the total amount extracted due to increasing solubility of the solute in the solvent.

(iv) Moving Vial: This means of agitation involves physically moving the vial. It is not widely used but gives good agitation of the sample. The main problem with this method is that it puts the fibre under a lot of stress. This can result in fibre breakage due to its intrinsic fragile nature.

(v) Fibre movement: Automated SPME achieves sample agitation by vibration of the fibre in the sample vial. Again this method puts the fibre under stress and it is limited to small sample volumes.

1.4.3 Desorption of the Analyte

Once the analytes have been adsorbed onto the fibre the next task is the quantitative transfer of these analytes into the analytical instrument and onto the column. This is done by placing the fibre in the hot injector port where thermal desorption is the means by which the adsorbed compounds are removed from the fibre, as shown in **Figure 1.4**.

Once again there are a number of variables that have to be considered. Ensuring the analytes are fully desorbed and that they leave in a narrow band are the two main problems that have to be addressed[35 36]. Factors influencing the desorption process which therefore affect these problems are

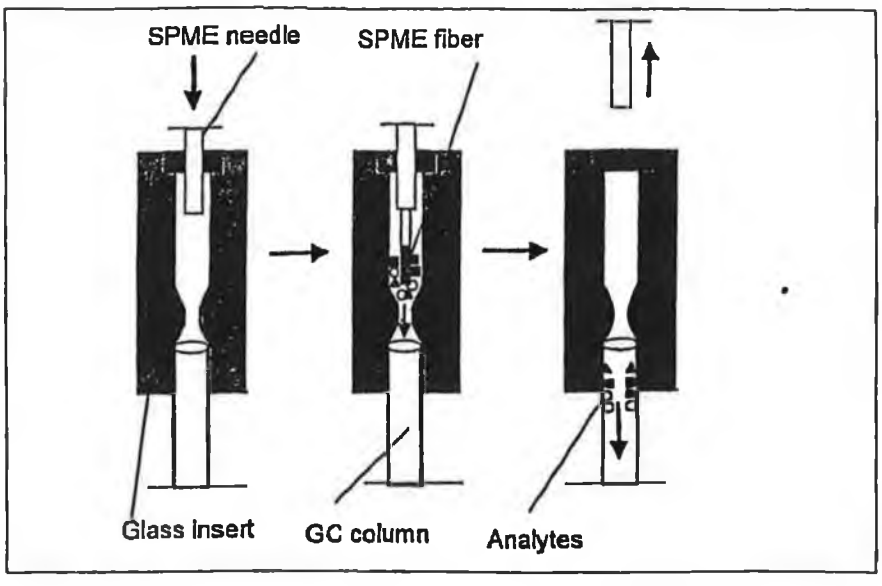

Figure 1.4: Desorption of the analytes[37]

(i) Desorption Temperature: This is the main factor in determining the desorption time. Many SPME users use the maximum desorption temperature that the fibre coating can withstand and then gradually increase the desorption time until no carryover is observed. Another approach is to increase the injector temperature until no carryover is observed on the fibre. This latter approach can result in a longer fibre coating lifetime as the maximum temperature which the coating can withstand may not be reached.

(ii) Linear flow rate around fibre: Standard chromatographic injectors, such as the popular split/splitless types, require large volume inserts to accommodate the expansion of the evaporated solvent introduced during injection. Large internal diameter (3-5mm) glass or fused silica tubes used for this purpose produce very low linear flow rates in the injector, resulting in a slow transfer of analytes onto the GC column. The split opening allows injection band sharpening and removal of remaining solvent vapours. In SPME introduction, no solvent is present and therefore the split is unnecessary. In fact, for optimum sensitivity, the split needs to be closed during desorption to transfer all analytes onto the column. To facilitate sharp SPME injection bands, the analytes desorbed should be removed rapidly from the injector. This can be accomplished by generating high linear flow rates of the carrier gas

around the fibre. Since volumetric flow rates are fixed at optimum operation of gas chromatographic separation (about 1ml/min), the practical way to achieve high linear flows is to reduce the diameter of the injector insert, matching it as closely as possible to the outside diameter of the fibre. For this reason special SPME injector liners with a very small internal diameter of 0.8 mm are available. As the linear flow rate around the fibre increases, the desorption time decreases and the analytes elute in a narrower band due to less dilution by the carrier / stripping gas. **Figure 1.5** demonstrates this principle.

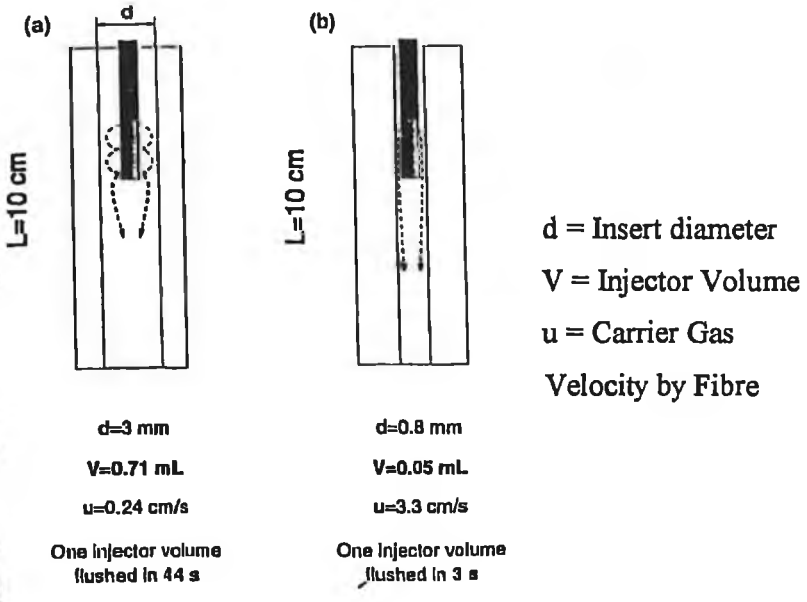

Figure 1.5: Effect of injector volume on the desorption process[2]

(iii) Fibre position in the injector: GC injectors have a temperature gradient associated with them – the middle of the injector being the hottest part (**Fig.1.6**). If the fibre is not placed in the same position in the injector each time, the temperature it experiences will be different. Reproducibility will then suffer. It is therefore important to find the hottest part of the injector and ensure the fibre is placed in this position for each run.

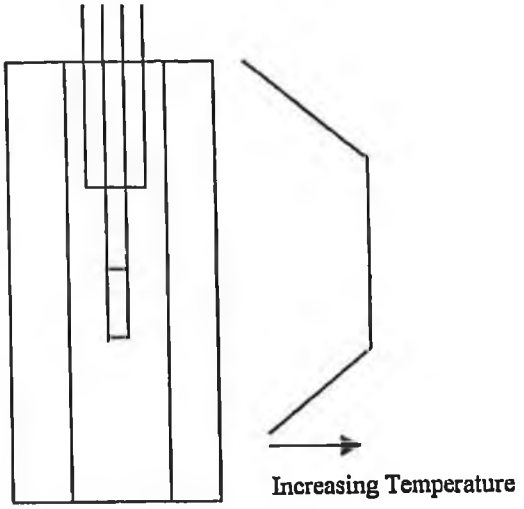

Figure 1.6: Typical injector temperature profile

(iv) Protecting Needle: The septum-piercing needle protecting the fibre is flat tipped, as opposed to tapered, to prevent damage to the fibre as it slides in and out of its protective casing. This flat-tipped needle is inclined to tear slivers from the septum as the septum is punctured. These slivers can subsequently become lodged in the liner or at the head of the column. Septa are manufactured from a silicone elastiomer which includes polydimethylsiloxane. As a result these small septum remains can act as an additional source of carryover and band-broadening as the organic analytes can become adsorbed on the fragments and released at a later stage.

Occasionally SPME related peak splitting associated with the protecting needle is observed. As soon as the needle enters the injector it starts to heat and some of the analytes begin to leave the fibre. When the whole fibre is exposed the bulk of the analytes become desorbed. If the time delay between the needle entering the injector and the fibre being exposed is too long a split peak will be observed – the first smaller peak corresponding to those molecules which left before the fibre was exposed and the second larger peak corresponding to the bulk which left upon exposure (**Figure 1.7**).

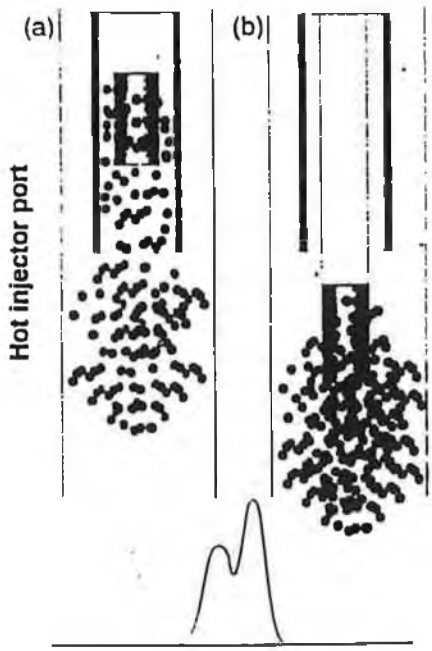

Figure 1.7: Diagram depicting how split peaks are formed as a result of slow fibre exposure in the injector[2]

1.4.4 Extraction Temperature

Increasing the extraction temperature results in shorter equilibrium extraction times as diffusion coefficients of the analyte within the system increase. However the coating/sample distribution coefficient decreases with an increase in temperature, resulting in a diminution in the equilibrium amount of analyte extracted[38]. In general, if the extraction rate is of major concern, the highest temperature which still provides satisfactory sensitivity, should be used. An internally cooled fibre SPME device would eliminate the sensitivity loss but this device is not yet commercially available. One internally cooled fibre design consists of a hollow fused silica fibre fused at one end and coated with the extracting phase[39]. Liquid carbon dioxide placed inside the fibre results in a coating temperature lower than that of the sample. This "cold finger" effect results in accumulation of analytes in the coating. In headspace SPME, an increase in extraction temperature also leads to an increase of analyte concentration in the headspace, and helps to facilitate faster extraction. Variation in the temperature at which the extraction takes place is a contributory factor to poor repeatability. Extraction temperature should be kept constant (ideally 18°C ± 2 °C).

1.4.5 Matrix modification

Salt concentration and sample pH control can be used to enhance extraction[40]. pH adjustment can improve sensitivity for basic and acidic analytes. For full conversion of acidic compounds to the neutral extractable form the pH should be at least two units below the pK_a of the analyte and for basic compounds two units above the pK_b value. When dealing with acidic or basic analytes the pH optimisation should include experimental verification of the expected results since adding a buffer to the sample modifies the matrix, which results in distribution constant changes. In practice, it can be difficult to implement pH change with the direct extraction approach as direct contact of the coating with high and low pHs damages the fibre coating. Headspace SPME can be used to overcome this problem for sufficiently volatile analytes.

Modification of the sample matrix by addition of salt can also enhance headspace extractions. This "salting out" technique causes the solubility of the organics in the aqueous sample to decrease forcing them into the headspace and subsequently the fibre coating, thus increasing the sensitivity of the method.

1.4.6 Selection of fibre coating

Table 1.1 shows the different types of fibres commercially available. Since only general coatings are available the selectivity which can be obtained is based primarily on polarity and volatility. Limits of detection will ultimately depend on the coating selected and equilibration times will vary depending on the coating thickness[41]. The maximum desorption temperature which a fibre can withstand varies with the phase, as does the conditioning times and temperatures. All fibres require careful handling due to their fragile nature but special care should be exercised when using certain types, in particular PDMS/DVB and Carbowax/DVB fibres as the slightest mechanical abrasion can result in these coatings being inadvertently stripped off.

Fibre coatings can be bonded, non-bonded or partially cross-linked. Chemical bonding and cross-linking inhibit bleeding of the coating. Bonding involves attaching a monomolecular layer of the stationary phase to the silica surface of the column by a

chemical reaction, the nature of which is proprietary. Cross-linking involves using a short side chain of atoms to link two longer chains in the polymeric material. Bonded phases are stable with all organic solvents but slight swelling may occur in some non-polar liquids. Non-bonded phases are stable with some water-miscible organic solvents, but may also swell. They should never be used or rinsed with non-polar organic solvents. Partially crosslinked phases are intermediate between bonded and non-bonded – they are stable in most water-miscible organics and may be stable in some non-polar solvents but again slight swelling may occur. Supelco, the manufacturers of the commercially available fibres, have published a number of Application Notes and Bulletins which can be consulted to determine the solvent / fibre compatibility and to select the most suitable fibre for a particular application. This literature can be accessed via Supelco's web site at www.sigma-aldrich.com.

Table 1.1: List of commercially available fibres, coating thicknesses, maximum operating temperatures and characteristic applications

Fibre Type	Coating Thickness (μm)	Max. Operating Temperature (°C)	Applications
PDMS	100	280 ° C	Volatile Analytes
PDMS	30	280 ° C	Non-polar Semivolatile Analytes
PDMS	7	340 ° C	Mid to Non-polar Semi-Volatile Analytes
PDMS/DVB	65	270 ° C	Polar Volatile Analytes
PA	85	320 ° C	Polar Semi-Volatile Analytes
Carboxen/PDMS	75	320 ° C	Very Small Volatile Analytes
Carbowax/DVB	65	265 ° C	Polar Analytes

The extraction time and the sensitivity of the method are determined both by the coating thickness and the distribution constants. In general, thinner coatings should be used for compounds with larger distribution constants. Whilst the use of thinner coatings will result in decreased equilibration times this is at the expense of lower sensitivity as they have a smaller analyte capacity due to smaller coating volume[38].

Polydimethylsiloxane is the most universal of the fibre coatings. It is rugged and should be used whenever possible. It comes in a number of thicknesses (100, 30 and 7μm) but the minimum thickness that achieves the required detection limits should be used as this will result in the shortest extraction times. PDMS is a nonpolar phase and extracts nonpolar analytes very well but it can also be applied to more polar compounds particularly after optimising extraction conditions[42].

For more polar analytes, such as phenols, polyacrylate is the coating of choice[37]. It is a low-density solid polymer at room temperature, which allows analytes to diffuse into the coating. Diffusion coefficients are lower than those for PDMS, resulting in longer extraction times.

Mixed phase coatings are also widely used in solid phase microextraction. Small analytes which diffuse rapidly are not well retained by liquid phases such as PDMS. Blending of a porous material like Carboxen with a liquid phase such as PDMS results in a primarily solid fibre coating which has the ability to more strongly retain analytes by physically trapping the analytes in the pores of the materials. The carboxen / PDMS fibre is more successful than the 100 μm PDMS fibre for the extraction of very volatile compounds such as dichlorofluoromethane, chloromethane, chloroethane and vinyl chloride[43 44]. Another mixed phase coating is the carbowax / DVB coating which is suitable for the extraction of polar volatile compounds such as alcohols[45], while the PDMS / DVB fibre is good for the extraction of semilvolatile compounds[46] while at the same time being compatable with the organic solvents used in HPLC[47].

1.4.7. SPME derivitisation techniques

Certain compounds are not very suitable for SPME – they may not be extracted by any of the available fibre coatings or, when extracted, may not be capable of being separated or detected chromatographically. By derivatizing the analyte one or both of these problems can be overcome – the derivatisation reaction may produce a specific derivative with sufficiently different volatility and polarity to allow extraction, or convert it to a form which is more amenable to chromatographic detection.

Derivatization can be carried out pre-extraction, simultaneously with extraction or post extraction. Derivatization performed before and/or after extraction can enhance sensitivity and selectivity of both extraction and detection, while post-extraction methods can only improve chromatographic behavior and detection of the analyte.

1. Pre-extraction derivatisation: The derivatising agent is added to the vial containing the sample. The derivatives formed are extracted by SPME and introduced into the analytical instrument. This approach has been applied to enable extraction of phenols from aqueous samples by first converting them to their acetate derivatives[37].

2. Post-extraction derivatisation: This involves exposing the fibre containing the extracted analytes to the derivatising agent. This approach has been successfully applied to the analysis of high molecular weight carboxylic acids. After exposing a SPME fibre containing the extracted carboxylic acids to diazomethane, the resulting ester derivatives can be separated as narrow bands on a GC column[48].

3. Simultaneous derivatisation and extraction in the fibre coating: Prior to extraction the fibre is doped with the derivatising reagent. This is done by first dissolving the reagent in a volatile solvent followed by immersion of the fibre in the solution. The fibre coating swells and is doped with the reagent, which after evaporation of the solvent is ready for use. A fibre that was doped with 1-pyrenydiazomethane was exposed to the headspace above a sewage sample in an experiment to detect volatile carboxylic acids. 1-pyrenediazomethane has sufficiently low vapour pressure and affinity towards the coating that it remains on the fibre during its exposure to the sample. Similarly the pyrenylmethyl esters formed also remain on the fibre until thermal desorption in the GC injector port. The process is no longer an equilibrium process as derivatised analytes are collected on the fibre for as long as extraction continues or until the analyte / reagent becomes exhausted. Consequently, doping of the fibres in this way with the derivatising agent facilitates not only spot sampling but also determination of long-term exposure to certain analytes. Volatile carboxylic acids were successfully detected in this way.

Direct SPME could not detect these carboxylic acids as selective films for these analytes have yet to be produced[49].

1.5 SPME - HPLC

SPME is rapidly becoming an accepted and widely used sample preparation technique for gas chromatography. Many compound classes, such as the weakly volatile polynuclear aromatic hydrocarbons[10], are more suited to analysis by HPLC and can be effectively extracted by solid phase microextraction. Transfer of the extracted analytes from the fibre to the HPLC column is not as simple as for gas chromatography as it requires a specially designed SPME / HPLC interface[50]. The interface, which is easily installed and removed, consists of a six-port injection valve and a desorption chamber that replaces the injection valve in the HPLC system. The SPME fibre is introduced into the desorption chamber under ambient pressure when the injector valve is in the "load" position. After the fibre is inserted through the ferrule, the unit is made leak-tight by closing a sealing clamp and compressing the ferrule against the SPME needle. All surfaces, which contact the SPME fibre or the mobile phase, are stainless steel or VESPEL®. Within the interface, mobile phase contacts the SPME fibre, removes the adsorbed analytes, and delivers them to the column for separation. Analyses to which SPME - HPLC have been applied include fat-soluble vitamins in tablets[51], explosives in water[52], PAHs[11] and pesticides[53].

1.6 THEORY OF SPME

An understanding of SPME theory identifies parameters for rigorous control and optimisation when developing methods, in addition to minimizing the number of experiments that need to be performed. To simplify mathematical relationships the theory assumes idealised conditions. Factors including thermal expansion of polymers, changes in diffusion coefficients due to the presence of solutes in polymers and heterogeneity of the matrix or the sorbing phase are all ignored. For simple matrices like air or drinking water the theory can very accurately predict extraction times and amounts extracted at equilibrium extraction time. For more complex conditions, theory for ideal conditions still approximates well general relationships

between parameters and extraction times or amounts extracted. The amount of analyte extracted at equilibrium conditions can be calculated using thermodynamic principles, while the extraction time can be estimated by solving differential equations describing mass transfer conditions in the system.

1.6.1 Theoretical determination of the mass of analyte extracted by a fibre coating

The initial analyte concentration in a system consisting of sample, fibre and headspace can be described as

$$C_O V_s = C_f^{\infty} V_f + C_h^{\infty} V_h + C_s^{\infty} V_s \qquad 1.1$$

Where

C_o = Initial concentration of the analyte in the matrix

C_f^{∞} = Equilibrium analyte concentration in the fibre coating

C_h^{∞} = Equilibrium analyte concentration in the headspace

C_s^{∞} = Equilibrium analyte concentration in the sample

V_f = Fibre Coating volume

V_h = Headspace volume

V_s = Sample volume

Defining the coating / gas distribution constant (K_{fh}) as $C_f^{\infty}/ C_h^{\infty}$ and the gas / sample matrix distribution constant (K_{hs}) as $C_h^{\infty}/ C_s^{\infty}$, the mass of analyte absorbed by the coating, $n = C_f^{\infty} V_f^{\infty}$ can be expressed as

$$n = \frac{K_{fh} K_{hs} V_f C_o V_s}{K_{fh} K_{hs} V_f + K_{hs} V_h + V_s} \qquad 1.2$$

The chemical potential of an analyte in the headspace can be expressed as

$$\mu_h = \mu^0 (T) + RT \ln (\rho_h / \rho_0) \qquad 1.3$$

where

μ_h = chemical potential of the analyte in the headspace

ρ_h = vapor pressure of the analyte in the headspace

$\mu^0(T)$ = chemical potential of the analyte at standard pressure and temperature T

R = universal molar gas consant

ρo = standard pressure (usually 1 atmosphere)

Similarly

$$\mu_f = \mu^0 (T) + RT \ln (\rho_f / \rho_0) \qquad 1.4$$

$$\mu_s = \mu^0 (T) + RT \ln (\rho_s / \rho_0) \qquad 1.5$$

where μ_f and μ_s are the chemical potentials of the analyte in the coating and the aqueous matrix,respectively; and ρ_f and ρ_s are the vapour pressures of the analyte in equilibrium with the analyte in the coating and the aqueous matrix, respectively.

When the three phase system is at equilibrium the chemical potentials of the analyte in all three phases must be equal, by definition.

$$\mu_f = \mu_h = \mu_s \qquad 1.6$$

therefore

$$\rho_f = \rho_h = \rho_s \qquad 1.7$$

According to Henry's Law

$$\rho_f = K_f C_f^{\infty} \qquad 1.8$$

$$\rho_s = K_s C_s^{\infty} \qquad 1.9$$

where K_f and K_s are Henry's Law constants of the analyte in the liquid polymer coating and the aqueous solution, respectively. Assuming that the ideal gas law

$$p_h V_h = n_h RT \qquad 1.10$$

(where n_h is the number of moles of the analyte in the headspace) is valid for the analyte vapour in the headspace, then

$$p_h = C_h^{\infty} RT \qquad 1.11$$

From equations 7 – 11 the distribution constants can be connected with Henry's constants, giving

$$K_{fh} = C_f^{\infty} / C_h^{\infty} = RT / K_f \qquad 1.12$$

$$K_{hs} = C_h^{\infty} / C_s^{\infty} = K_h / RT \qquad 1.13$$

In the case of direct SPME sampling of an aqueous solution at equilibrium $\mu_f = \mu_s$ and $\rho_f = \rho_s$. K_{fs} can be expressed as $C_f^{\infty} / C_s^{\infty} = K_h K_f$ since $\rho_f = K_f C_f^{\infty}$, $\rho_s = K_h C_s^{\infty}$ and $\rho_f = \rho_s$ when equilibrium is reached. Based on equations 12 and 13, then

$$K_{fh} = K_H / K_F = K_{fh} K_{hs} = K_{fg} K_{gs} \qquad 1.14$$

Equation 2 can then be rewritten as

$$n = \frac{K_{fs} V_f C_o V_s}{K_{fs} V_f + K_{hs} V_h + V_s} \qquad 1.15$$

This equation states that the amount of analyte extracted is independent of the location of the fibre in the system assuming equilibrium has been reached within the system. It may be placed in the headspace or directly in the sample as long as the volume of the fibre coating, headspace and sample are kept constant.

Assuming that the vial is full and no headspace exists equation 15 simplifies to

$$n = \frac{K_{fs} V_f C_o V_s}{K_{fs} V_f + V_s} \qquad 1.16$$

as the term $K_{hs} V_h$, representing the capacity ($C_h^{\infty} V_h$) of the headspace, can be eliminated.

The sensitivity of the SPME method is proportional to the number of moles of analyte, n, extracted from the sample. As the sample volume increases so also does the amount of analyte extracted (Equation 16), until the volume of the sample becomes significantly larger than the product of the fibre/sample distribution constant and volume of the coating i.e. until $K_{fs}V_f << V_s$. For compounds with a K_{fs} up to about 200 (typical of many of the volatile organic compounds) and a 100 μm PDMS fibre, a 2 ml vial is sufficient to give maximum sensitivity

Equation 16 describes that situation when SPME is said to be complete. In practice this means that once equilibrium has been reached, the extracted amount is constant within the limits of experimental error and it is independent of further increases in extraction time. Although all these discussions have been limited to equilibrium situations it must be noted that the extraction can be interrupted and the fibre analysed prior to equilibrium. To obtain reproducible data, constant convection conditions and, as stressed later, careful timing of the extraction are necessary.

1.6.2 SPME Kinetics

The kinetics of the extraction process determines the speed of the extractions. Kinetic theory identifies "bottlenecks" of solid phase microextraction and indicates strategies to increase speed of extractions. Most of the theory of mass transfer from the solution into the fibre is based on Fick's Second Law. Fick's laws of diffusion, of which there are two, model the diffusion process. Ficks First Law states that the rate of diffusion of a species in a given direction is proportional to the concentration gradient in that direction, i.e.

$$J = -D\,(dc/dx) \qquad 1.17$$

where J = the rate of diffusion of a species

D = diffusion coefficient

dc/dx = the concentration gradient

The first law is combined with a continuity equation, and the assumption that D is a constant, to give the second law which states that the rate of change of concentration with time is proportional to the change in concentration gradient with distance in a

given direction. Mathematical solutions and derivations can be found in reference two.

Taking the simplest system whereby a fibre is placed in a water sample that is perfectly agitated i.e. there is no concentration gradient within the sample. Before the fibre is placed in the solution, no analyte is present in the coating. Immediately after immersion into the sample, only a thin layer close to the surface contains analyte. With time, analyte molecules diffuse progressively deeper into the coating and eventually reach equilibrium. This has been shown experimentally, as under exactly the same conditions and for extraction of the same analyte, the equilibrium extraction time for a 7μm PDMS fibre is shorter than for a 100 μm thickness of the same coating. For this reason the fibre with the thinnest coating that can achieve the required limits of detection should be used.

Independent of the agitation level, fluid contacting a fibre's surface is always stationary. As the distance from the fibre surface increases, the fluid movement gradually increases until it corresponds to bulk flow in the sample. The static layer contacting the fibre's surface is called a Prandtl boundary layer. The agitation conditions and the viscosity of the fluid determine its thickness. Better agitation of the sample results in a decrease in the thickness of the Prandtl boundary layer.

Before the fibre is placed in the solution no analyte is present in the fibre coating. The concentration in the Prandtl layer is the same as in the bulk solution. After immersion in the sample, as in the perfectly agitated case, most of the extracted analyte is present in a thin layer of the coating close to the surface. Now the concentration in the aqueous phase close to the fibre surface substantially decreases and a concentration profile is formed in the Prandtl layer. This results in lower concentration gradients in the coating at the interface with the fibre surface and slower mass transport into the system. The effect of the boundary layer size on the equilibration rate is quite pronounced. The equilibration time for a perfectly agitated sample is 20 seconds, compared to 25 seconds for a 10 μm boundary layer and 95 seconds for a 100 μm boundary layer. A more detailed treatment on the dynamics of

organic compound extraction from water using liquid-coated fused silica fibres can be found in reference 33.

1.7 FIBRE CARE

A variety of factors may influence the fibre response or absolute amount extracted. These include coating of the fibre surface with sample matrix components, physical damage or deterioration of the fibre coating and lot-to-lot or fibre-to-fibre variation in coating character or length. Even headspace analyses have been observed to result in a coating of the fibre with matrix components after many extractions. An electron micrograph of a 75μm Carboxen / PDMS fibre (**Figure 1.8**) shows the porous nature of the coating. The tip of the fibre can be seen to be clearly damaged. The fibre not being retracted far enough inside the protecting needle when puncturing the injector or sample vial septum most likely caused this damage.

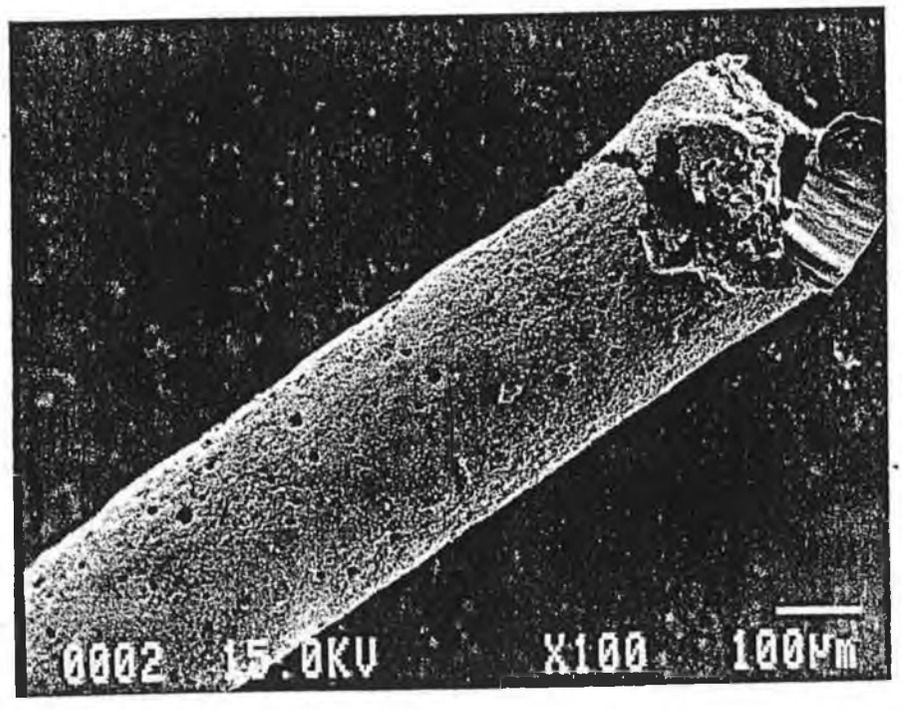

Figure 1.8 Electron micrograph of a 75μm Carboxen / PDMS fibre showing damaged tip

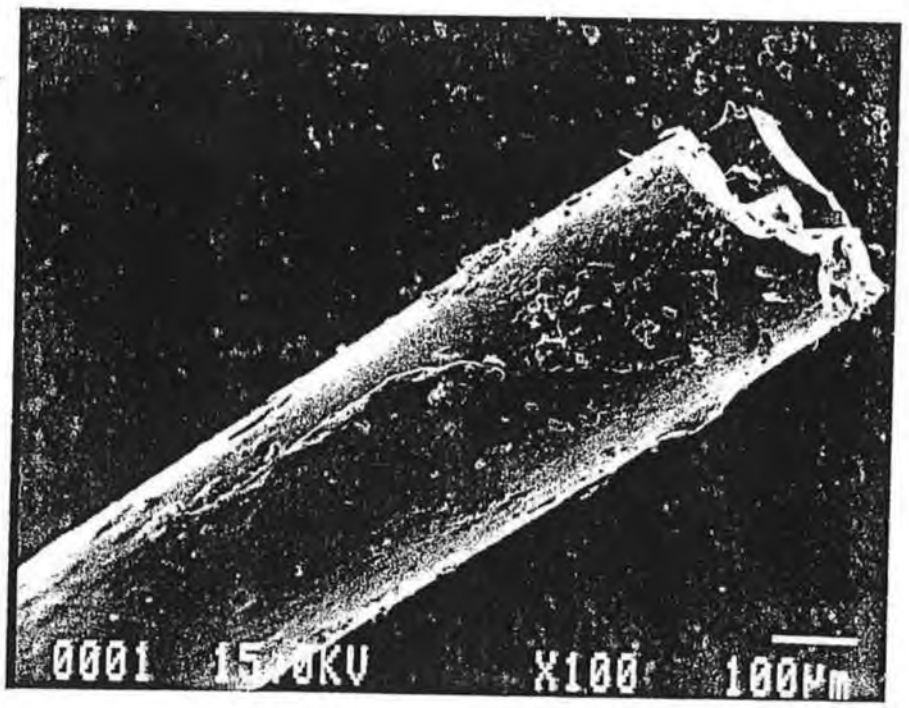

Figure 1.9 Electron micrograph of 100µm PDMS fibre showing surface fouling

Figure 1.9 shows a soiled 100µm PDMS fibre. This fibre had been used to extract VOCs from various water samples. Organic material adhered to the surface of the fibre is visible. This fibre had been conditioned at the recommended conditioning temperature of 250°C for 1 hour prior to microscopic examination. Where conditioning does not remove contaminants, cleaning may be carried out by soaking in water or an appropriate solvent (eg. methanol) while stirring. Fibres may then be dried, typically for a few hours or overnight, and reconditioned at the recommended temperature for a short time before the next use. Visual inspection of the fibre using an illuminated magnifying glass will identify those samples causing fibre fouling and an appropriate step such as rinsing with purified water can be employed between extractions.

1.8 CONCLUSION

Solid phase microextraction is a sample preparation technique which can be used to extract a range of organics with little perturbation of the sample – the amount extracted is negligible compared to the overall analyte concentration in the sample. Analytes partition into an adsorbent material coated on a fused silica fibre and are most often subsequently thermally desorbed in the heated injection port of a gas chromatograph. It is solvent free and rapid. Factors such as extraction time, extraction mode, desorption time and temperature, extraction temperature, and fibre coating amongst others affect precision, accuracy, repeatability and efficiency of the extraction.

Applications of SPME are numerous. Initially used in extraction of environmental samples this versatile technique has also found applications in food, forensic and biomedical analysis. Although solid phase microextraction was originally named after microextraction using the coated fused silica fibres described hereto new configurations are expected to emerge in the future. Such configurations are expected to include coated vessels, agitation disks and suspended particles. Another possibility is the use of coated tubes through which the sample can flow. Exciting possibilities also exist for development of new fibre coatings including coatings incorporating antibodies for very specific extractions. Attention must also be given to quality control procedures used in the manufacture of the fibres as it has recently been shown that some reproducibility problems experienced during analysis can originate from variable surface properties of different fibres[54].

1.9 REFERENCES

[1] C.L. Arthur and J. Pawliszyn, Anal. Chem., **62** (1990) 2145.

[2] J. Pawliszyn, "Solid Phase Microextraction: Theory and Practice", Wiley, New York (1997).

[3] Z. Zhang, J.Poerschmann and J.Pawliszyn, Anal. Chem., **33** (1996) 219.

[4] Federal Register, "Rules and regulations, method 625, base/neutrals and acids", **49** (1984), 43385.

[5] Leaflet accompanying each pack of fibres, Sigma Aldrich, 1996

[6] R.G. Belardi and J. Pawliszyn, Water Pollut. Res. J. Can., (1989) 179.

[7] T. Gorecki and J. Pawliszyn, J. High Resol. Chromatogr., **18** (1995) 161.

[8] T. Nilsson, F. Pelusio, L. Montanarella, B. Larsen, S. Facchetti and J.O. Madsen, J. High Resol. Chromatogr., **18** (1995) 617.

[9] Y. Yang, D.J. Miller, S.B. Hawthorne, J. Chromatogr. A, **800** (1998) 257.

[10] S. Magdic and J. Pawliszyn, J. Chromatogr. A, **723** (1996) 111.

[11] J. Chen and J. Pawliszyn, Anal. Chem., **67** (1995) 2530.

[12] K.J. Hageman, L. Mazeas, C.B. Grabanski, D.J. Miller and S.B. Hawthorne, Anal. Chem., **68** (1996) 3892.

[13] R. McCallum, P. Pendleton, R. Schumann and M.U. Trinh, Analyst, **123** (1998) 215.

[14] M. Winkler, J.V. Headley and K.M. Peru, J. Chromatogr. A., **903** (2000), 203.

[15] M. Chai and J. Pawliszyn, Environ. Sci. Technol., **29** (1995) 693.

[16] J. Koziel, M.Y. Jia and J. Pawliszyn, Anal. Chem, (2000) 5178.

[17] M. Guidotti, R. Giovinazzo, O. Cedrone, M. Vitali, Environment International, **26** (2000), 23.

[18] L. Pan, J.M. Chong and J. Pawliszyn, J. Chromatogr. A., **773** (1997) 249.

[19] P.A. Martos, A. Saraulio and J. Pawliszyn, Anal. Chem. **69** (1997) 402.

[20] C. Malosse, P. Ramirez-Lucas, D. Rochat and J.P. Morin, J. High Resol., Chromatogr. **18** (1995) 669.

[21] B. Schafer, P. Hennig and W. Engewald, J. High Resol. Chromatogr., **18** (1995) 587.

[22] D.C. Robacker and R.J. Bartlet, J. Agric. Food Chem., **44** (1996) 3554.

[23] H. Lord and J. Pawliszyn, J. Chromatogr. A, **902** (2000) 17.

[24] S. Ulrich, J. Chromatogr. A, **902** (2000) 167.

[25] G.A. Mills and V. Walker, J. Chromatogr. A, **902** (2000) 267.

[26] K.G. Furton, J.R. Almirall and J.C.Bruna, J. Forensic Sci., **41** (1996) 12.

[27] X.P. Lee, T. Kumazawa, K. Sato, H. Seno, A. Ishii, O. Suzuki, Chromatographia, **49** (1998) 9.

[28] G. Fitzgerald, K.J. James, K. MacNamara and M.A. Stack, J. Chromatogr. A, **896** (2000) 351.

[29] L.K. Ng, M.Hupe, J. Haronis and D. Moccia, J. Sci. Food Agric., **70** (1996) 380.

[30] D. De La Calle Garcia, S. Magnaghi, M. Reichenbaecher and K. Danzer, J. High Resolut. Chromatogr., **19** (1996) 257.

[31] Z. Penton, Food Test. Anal., **2** (1996) 16

[32] M. Chai, C.L. Arthur, J. Pawliszyn, R.P. Belardi and K.F. Pratt, Analyst, **118** (1993) 1501.

[33] S.D. Huang, C.P. Cheng and Y.H. Sung, Analytica Chemica Acta, **343** (1997) 101.

[34] D. Louch, S. Motlagh and J. Pawliszyn, Anal. Chem., **64** (1992) 1187.

[35] N.H. Snow and P. Okeyo, J. High Resol. Chromatogr., **20** (1997) 77.

[36] J.J. Langenfield, S.B. Hawthorne and D.J. Miller, J. Chromatogr. A, **740,** (1996), 139.

[37] J.A. Koziel and J. Pawliszyn, J. Air and Waste Manage. Assoc., **51** (2001) 173.

[38] Z. Zhang, M.J. Yang and J. Pawliszyn, Anal. Chem., **66** (1994) 844.

[39] Z. Zhang and J. Pawliszyn, Anal. Chem., **67** (1995) 34

[40] K.D. Buchholz and J. Pawliszyn, Anal. Chem., **66** (1994) 160.

[41] F.J. Santos, M.T. Galceran and D. Fraisse, J. Chromatogr. A, **742** (1996) 181.

[42] J. Dugay, C. Miege and M.C. Hennion, J. Chromatogr. A, **795** (1998) 27.

[43] P.Poppe and A. Paschke, Chromatographia, **46,** (1997), 419.

[44] R.E. Shirey and W.R. Betz, "SPME:Using porous Materials as Fiber Coatings", Presented at the Canadian Chemical Society Meeting, June 1997, ©Sigma-Aldrich Co.

[45] R. Shirey, V. Mani and M. Butler, Supelco Reporter, **14** (1996) 4.

[46] H.A. Lakso and W.F. Ng, Anal. Chem., **69**, (1997), 1866.

[47] G. Gora-Maslak, Supelco Reporter, **15** (1996) 1.

[48] L.Pan And J. Pawliszyn, Anal. Chem., **69** (1997) 196.

[49] L. Pan, M.Adams and J. Pawliszyn, Anal Chem., **67** (1995) 34.

[50] J. Chen and J. Pawliszyn, Anal. Chem., **67** (1995) 2530.

[51] G. Gora-Maslak and R. Mindrup, Supelco Reporter, **16** (1997) 10.

[52] G. Gora-Maslak, Supelco Reporter, **15** (1996) 5.

[53] G. Gora-Maslak and V. Mani, Supelco Reporter, **16** (1997) 5.

[54] C.T. Harberhauer, M. Crnoja, E, Rosenberg and M. Grasserbauer, Fresnius J. Anal. Chem., **366** (2000) 329.

Chapter 2

Solid-phase Microextraction and its application to the analysis of Volatile Organic Compounds

2.1 INTRODUCTION

Water is the most common substance on earth and without it there can be no life. Industry uses more water than any material[1]. This along with other factors has resulted in one of our most valuable resources becoming contaminated with pollutants, causing problems for humans and other organisms dependent on water. One of the most common environmental pollutants are a family of compounds known as the Volatile Organic Compounds (VOCs). These compounds, with boiling points up to 200 °C and molecular masses in the range ca. 16-250 amu, are insoluble or only slightly soluble in water[1].

2.1.1 Toxicity of Volatile Organic Compounds

Many volatile organic compounds are mutagens, teratogens or carcinogens which are resistant to microbial or photochemical degradation[2]. Some organic compounds which enter the body tend to accumulate in fatty tissues[2]. It has been reported that carcinogenic and mutagenic effects have been induced in animals by several volatile halocarbon compounds[3]. Several studies have confirmed the carcinogenicity of chloroform in mammals[4 5]. Chloroform and other trihalomethanes are harmful to the liver, kidneys and blood[6]. Exposure to aromatic hydrocarbons can also cause serious health problems. Benzene is known to cause leukaemia[7]. The toxic properties of toluene, ethylbenzene and xylenes have also been frequently studied[8 9]. Some volatiles, especially polar compounds, are responsible for the degradation of organoleptic attributes such as taste and odour of the water, thereby affecting its quality[10].

2.1.2 Sources of contamination by volatiles

The sources of pollution by volatile organic compounds can be divided according to their origin into two basic groups

(i) Human activity e.g. municipal waste, traffic, industrial and agricultural sources;

(ii) Naturally occurring sources.

Man made sources: From municipal sources, various commercial products containing organic solvents contribute to pollution. These are one source of volatile organics (benzene, toluene, ethylbenzenes and xylenes – BTEX) and halogenated volatiles in the environment. Another source of halogenated volatiles is the treatment of water with chlorine, carried out in order to prevent outbreaks of water-borne diseases.

Traffic causes an increased content not only of some toxic gases such as CO and NO_x in the environment, but also of VOCs as a result of complex reactions during the combustion of petrol in vehicles[2]. VOCs can be successively transported from the air via rainwater into the aquatic environment[11]. Another important source of contamination is the leakage of petrol and diesel fuel from under-ground storage tanks. Accidents during the transport of petroleum products on giant sea tankers and during their manipulation in ports also contribute to pollution and consequently the extensive contamination of groundwaters near ports is not surprising.

Gaseous emissions from many industrial sources contain VOCs which are transported by rain or airborne particles to the water or soil. VOCs can be found in aquifers as a result of accidents which occur during storage and transport, diffuse seepage into the soil during their use by companies, and leaching in municipal landfills from household products such as cleaners and adhesives[12]. One large industrial source of volatile compounds are wastewaters from the petrochemical industry which contain large amounts of benzene, toluene, ethylbenzene and xylene (BTEX).

Pesticides are a source of agricultural pollution. They are often diluted with petroleum distillates that also contain BTEX. BTEX can be washed from soil by rainwater and transported to the aquatic environment. Sewage sludges, which have been added in many regions to agricultural soils to increase the organic content of the

soil, have a high adsorption capacity for VOCs. These compounds can be extracted from these sludges by rainwater and transported to water tables.

Naturally occurring sources: Organic pollutants in water can also originate from nature. Volatile aromatic compounds have been known since 1966 to be naturally occurring in soil. These aromatic compounds come from breakdown products of humic and fulvic acids which contain benzene in their structure and are naturally occurring in soil and water[13]. In addition to aromatics, naturally occurring non-aromatics are known e.g. monochloromethane, dichloromethane, monobromomethane, tribromomethane and chloroform are metabolic by-products products of marine organisms[6].

2.1.3 Techniques used in the analysis of Volatile Organic Compounds

Techniques used in the analysis of volatile organic compounds in water can be subdivided into the following groups

2.1.3.1 Gas extraction

The determination of VOCs in water samples at the part per billion (ppb) level requires the preconcentration of volatiles prior to GC analysis. One possible way is to use the gas as the extraction agent. Two modes of gas extraction can be distinguished: static headspace and dynamic headspace.

During static headspace the analytes become concentrated in the headspace above the sample in a sealed system. A needle can then be introduced to the headspace, a volume removed and injected onto the gas chromatograph. This technique is extremely simple but suffers from relatively low sensitivity. As a result, static headspace is suitable for the analysis of samples with higher contents of volatiles such as waste or municipal waters.

In the dynamic headspace method, the gaseous phase over the sample is permanently purged with carrier gas, which carries the analytes to the trapping medium. This

further pre-concentration on the trap allows for lower detection limits to be achieved. There are two variations of the dynamic headspace method: open-loop and closed-loop stripping.

Open-loop stripping involves passing the stripping gas through the sample and the trap and subsequently venting it to the atmosphere. The trapped analytes are thermally desorbed from the trap and swept onto the column. Closed-loop stripping differs from open-loop in that the stripping gas is recycled through the sample and trap as opposed to venting to the atmosphere. Although the instrumentation required is more complex, detection limits are better as the sample is exhaustively stripped of the analyte.

2.1.3.2 Liquid extraction

Another way to preconcentrate volatiles from water samples is to extract them into a small volume of organic solvent. The advantages of this procedure are its simplicity, speed, few requirements for special equipment and low solvent consumption. With one injection it is possible to analyse a wide range of compounds, not only volatile compounds. Disadvantages of this technique are the possible solvent co-elution with some of the more volatile compounds and the presence of analyte and/or other interfering compounds in the solvent.

2.1.3.3 Distillation techniques

Isolation of polar substances by many preconcentration techniques can be difficult; however, distillation is especially suitable for polar substances. Steam distillation and vacuum distillation are the two main distillation techniques used.

2.1.3.4 Direct injection

Direct Injection as the name implies involves injecting the sample directly onto the GC column. Whilst this procedure is rapid and simple, the contamination of the injector and GC column with sample matrix is a serious disadvantage. This technique has been applied mainly to the analysis of halogenated compounds in water using gas chromatography with electron capture detection.

2.1.3.5 Solid phase microextraction

Solid-phase microextraction (SPME) is one of the newest approaches to the analysis of volatile compounds. The operation of SPME has already been described in Chapter One. Although SPME methods are still in the relatively early stages of development, much work has been carried out on the application of SPME to the analysis of VOCs. Arthur and Pawliszyn published one of the first papers on this topic in which they describe how a chemically modified fused silica fibre can be used to extract organics from water and rapidly transfer the extract to a capillary column[14]. Since then many publications have described applications of SPME to a range of compounds, but particularly volatiles.

A considerable amount of work has been carried out on solid phase microextraction of volatile organic compounds from water. Much of this work explores its solvent free feature, speed of extraction and convenient automation and hyphenation with analytical instruments. Arthur *et al.* used SPME in the analysis of environmental water samples for organic compounds listed in EPA methods 624 and 524.2[15]. These compounds range from chloromethane to 1,1,2,2,-tetrachloroethane. The influence of stationary phase, injector, detector and stirrer on method development are discussed. This group found that with an ion-trap mass spectrometer, detection limits required by method 524 could be met for all compounds except chloromethane and chloroethane.

Zhang and Pawliszyn extracted BTEX and volatile chlorinated compounds from various matrices such as water, sand, clay and sludge with good precision and low limits of detection[19]. They reported that the sensitivity of their method could be greatly improved by the addition of salt to water samples or by heating. Another study published by Chai *et al.* on SPME of volatile chlorinated hydrocarbons concluded that their method was comparable to US EPA methods 502.2 and TO-14, achieving limits of detection in the parts per trillion range[16].

An evaluation of solid phase microextraction for analysis of volatile organic compounds carried out by Nilsson *et al.* found that for the sixty compounds studied

typical detection limits ranged from 20ng/l to 200ng/l, with the exception of the very light VOCs (Chloromethane, Vinyl Chloride, Bromomethane, 1,1- Dichloroethene and Methylene Chloride) for which detection difficulties were encountered[17]. The precision of analysis, 3% average standard deviation when an internal standard was used, was satisfactory for most routine quantitative applications. Extractions carried out at different temperatures found that while at increased temperatures the equilibration times decreased, the sensitivity of the method also decreased.

Environmentally hazardous samples of wastewater discharges were analysed for VOCs using SPME by James and Stack[12]. Fibres coated with either polydimethylsiloxane (PDMS) or polyacrylate were examined to determine VOCs which included chloroform, saturated carboxylic acids, alkyl-benzenes, phenol, benzonitrile and benzofuran. Detection limits varied from 10 to 170 μg/l and satisfactory relative standard deviations were obtained (RSD of <10%). For most samples headspace sampling was preferred to immersion sampling.

The continuous monitoring of levels of organic compounds in process streams and wastewater effluents is of particular importance to industry. Although this can be accomplished by using specific sensors, this approach is difficult to implement in practice because of the complexity of real samples. Motlagh and Pawliszyn have proposed an alternative scheme which interfaces gas chromatography to the analytical system using a simple SPME device[18].

In addition to the examples of various applications discussed, an inter-laboratory validation of solid-phase microextraction for the quantitative analysis of volatile organic compounds in aqueous samples was conducted by Nilsson *et al.* in which twenty laboratories participated[19]. Thirteen VOCs were used, ranging in volatility from vinyl chloride to 1,2- dichlorobenzene. The statistical evaluation of the accuracy and precision of SPME was performed in accordance with the ISO standard for inter-laboratory studies. Reference material were used and a comparison with the usual purge and trap and static headspace techniques were carried out. Comparable repeatability, reproducibility and accuracy were found between the SPME method and

the reference methods. Better precision of SPME was obtained by extraction from a small headspace over the aqueous phase than with the fibre immersed in water. The linearity was good in all cases and detection limits were in the low ng/l range. It was therefore concluded that SPME is a valid alternative to purge and trap and static headspace for the analysis of VOCs at trace levels in various aqueous samples.

2.1.4 Scope of this work

The US EPA method 524.2 which utilises purge and trap – gas chromatography – mass spectrometry for the analysis of Volatile Organic Compounds is a widely used and well established method in environmental monitoring. This chapter investigates the use of automated SPME as an alternative to purge and trap for the analysis of the sixty compounds on the EPA method 524.2 list. Environmental samples, typical of those subject to regulatory control, were analysed. These samples mainly consisted of discharges from pharmaceutical and landfill sites.

EU council directive 98/33/EEC 1998 states that member states shall take all measures necessary to ensure that regular monitoring of the quality of water for human consumption is carried out, in order to check that water available to consumers meets the requirements of this directive which is currently set at 100 µg/l for total trihalomethanes. In addition to the analysis of environmental samples this chapter also contains results from the analysis of mineral waters and treated tap water from local authority water supplies for total trihalomethanes (chloroform, bromoform, bromodichloromethane and chlorodibromomethane) using a SPME fibre coated with a 100 µm thick stationary phase of poly(dimethylsiloxane).

2.2 EXPERIMENTAL

2.2.1 Reagents and Materials

100 μm PDMS and 75 μm Carboxen / PDMS fibres were purchased from Supelco, Bellifonte, USA.

A calibration standard containing the complete list of US EPA 524.2 list VOCs was purchased from Supelco, Bellifonte, USA, as was the internal standard fluorobenzene. For trihalomethane analysis a standard was purchased from Supelco, Bellifonte, USA.

The water used throughout for preparation of standards was purified by passage through a carbon filter attached to a water cooler. This water, in addition to HPLC water and water from a Millipore system, was analysed by purge and trap – gas chromatography – mass spectrometry and was found to contain fewer traces of organic compounds than the other two sources.

Analysis of water samples for VOCs were carried out on wastewater dicharges from various pharmaceutical plants. These samples were obtained from the Environment Department, Enterprise Ireland. Due to most of these samples not containing VOCs it was necessary to fortify them beforehand. An intermediate standard was prepared in methanol from the VOC standard, and various volumes of this standard injected into the water sample to give the desired concentration. In order to minimise evaporation of the most volatile components the sample was placed in a rubber capped flask and the injection made through this cap.

For trihalomethane analysis commercially available mineral water samples were purchase in two different retail outlets. Water samples from local authorities were collected from domestic supplies at a number of different locations.

2.2.2 Apparatus

(a) Manual SPME was carried out using either a 100μm PDMS fibre or a 75μm carboxen/PDMS fibre attached to a Supelco SPME holder. For automated SPME the fibre was fitted to a Varian 8200 autosampler. 0.8ml of water was placed in a 2ml autosampler vial, capped and allowed to equilibrate for 5 minutes. The headspace was then extracted followed by thermal desorption of the fibre in the GC injector port for 0.7 minutes at 220°C and 250°C for PDMS and carboxen/PDMS fibres, respectively. The GC injector port was fitted with a low volume injector insert to improve chromatography and splitless injection was used.

(b) Gas chromatography – mass spectrometry was carried out using a Varian Star 3400 CX gas chromatograph interfaced to a Varian Saturn II ion-trap mass spectrometer. The gas chromatograph was fitted with a 30-metre × 0.25 mm internal diameter, 0.25μm phase loading, J+W DB-624 capillary column. The oven was initially held isothermally at 35 °C for 5 minutes, temperature programmed at 6 °C per minute to 125 °C, followed by 15 °C per minute to 220°C and finally held at 220 °C for 1.67 minutes - total run time 28 minutes. The mass spectrometer was scanned at 0.7 seconds / scan and monitored the mass range 35 – 650 a.m.u. The GC-MS transfer line temperature was 240 °C

For trihalomethane analysis the oven was initially held isothermally at 35°C for 5 minutes, and temperature programmed at 6°C per minute to 125°C - total run time 20 minutes.

(c) The purge and trap model used was a Tekmar LSC 2000 with a Tekmar ALS 2016 autosampler. Helium was used as the purge gas. The sample was purged for 11 minutes using a flow rate of 1 ml/min. Analytes were trapped on a Vocarb trap before being flash heated to 260°C for 1 minute

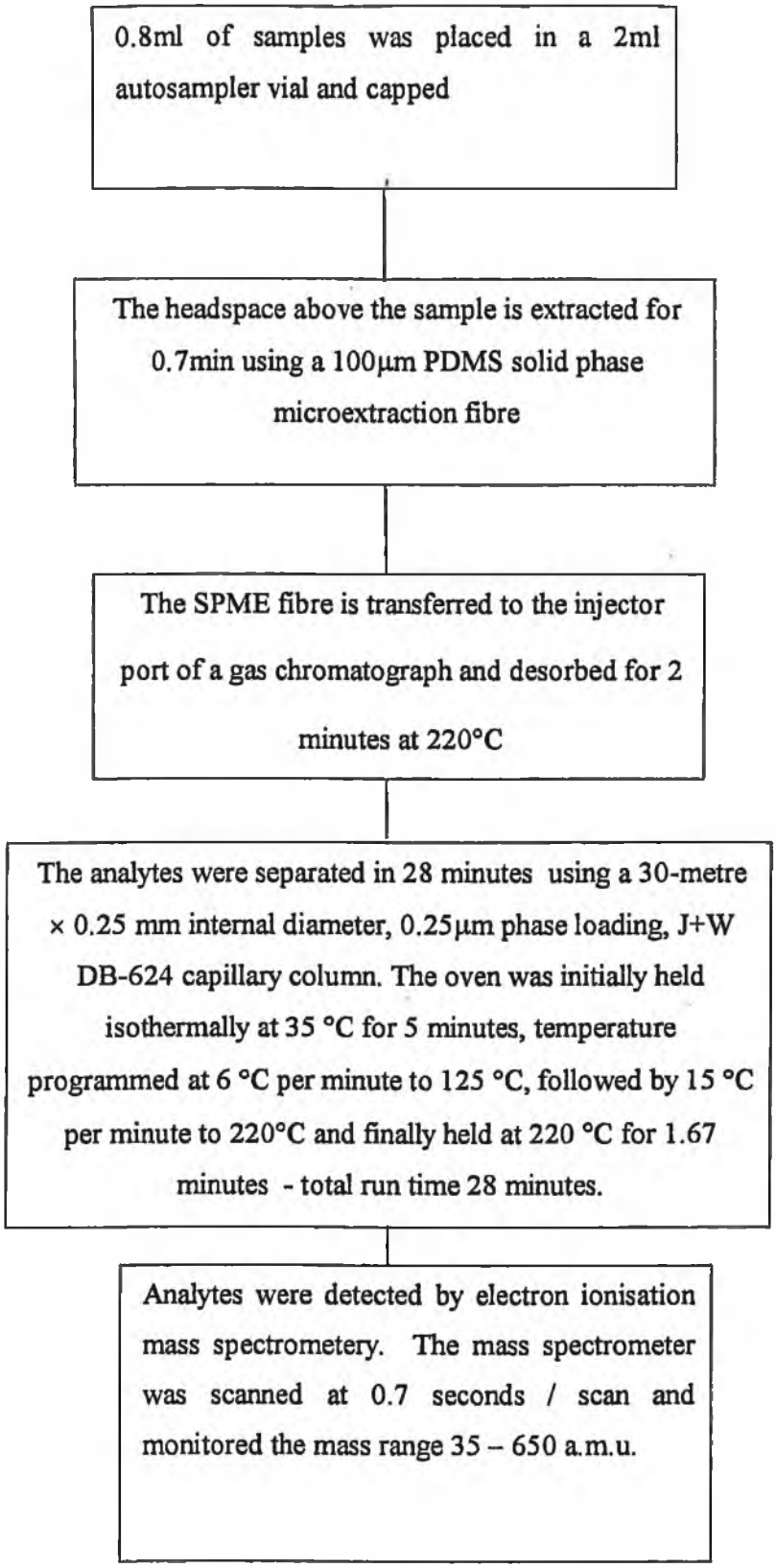

Figure 2.1: Summary of SPME method developed for the analysis of VOCs

2.3 RESULTS AND DISCUSSION

Solid-phase microextraction of organic compounds from water can be carried out in either the immersion or headspace modes. As the compounds under investigation are sufficiently volatile, headspace SPME is the preferred mode. Equilibrium extraction times are shorter using headspace sampling and soiling of the fibre from heavily contaminated samples is prevented, as there is no direct contact between fibre and sample. All results were therefore obtained using headspace solid phase microextraction.

2.3.1 Development of analysis conditions

The column temperature programme, carrier gas flow rate and mass spectrometer conditions optimised for Purge and Trap analysis was used also for SPME analysis as SPME is essentially a sample preparation and introduction technique and is not influenced by these parameters.

An in-house method developed in the Trace Organics Laboratory, Enterprise Ireland found that effective separation of the 60 VOCs could be achieved using those conditions outlined in **Section 2.2.2**.

In order to ensure the mass spectrometer was functioning properly a tuning compound was run on a daily basis. The tuning compound chosen was bromofluorobenzene (BFB) as this is the tuning compound recommended by the US EPA for volatile GC analysis. The mass spectrometer is deemed not to be functioning properly if the BFB spectrum does not pass the strict criteria outlined – See **Appendix One.**

2.3.2 Determination of optimum extraction time

Solid phase microextraction consists of two processes:

(i) Extraction of the analytes from the sample into the coating and

(ii) Desorption of concentrated analytes into the analytical instrument.

The rate at which analytes are extracted from the sample into the coating is dependent on transfer of the analyte through the sample matrix to the fibre surface and diffusion of the analyte into the coating. When the speed of extraction is solely determined by diffusion of analyte into the fibre coating equilibrium extraction time is very rapid i.e. generally less than one minute. The equilibrium extraction time is the time after which the amount of extracted analyte remains constant and corresponds within experimental error to the amount extracted at infinite extraction time. Ideally the equilibrium extraction time should be used, except where it is prohibitively long in which case the duration of the GC run should determine the extraction time i.e. when one sample is being analysed, another sample can be extracted. In practice short equilibrium extraction times can be achieved for extraction of gaseous samples due to large diffusion coefficients of organic compounds in air. For aqueous samples diffusion of the analyte through the matrix contributes significantly to the equilibrium extraction time and consequently on the amount of analyte extracted with time.

Investigations were carried out to determine the optimum extraction time. Three of the less volatile compounds on the EPA's 524.2 VOC list were chosen for this study – toluene, isopropylbenzene and hexachlorobutadiene. These compounds were chosen as their equilibrium extraction times are longer than more volatile compounds and so they will be the limiting compounds in the determination of the overall extraction time necessary for this analysis.

The headspace above a 10 ppb VOC standard was extracted for varying lengths of time (30, 60, 90, 120 and 300 seconds) using a 100 μm PDMS fibre followed by desorption at 250 °C for 2 mins. The extraction profile shown in **Figure 2.2** was obtained.

The extraction time profile is characterised by an initial rapid rise in the amount of analyte extracted by the fibre during the first minute. This is due to partitioning of those analytes originally present in the gaseous headspace, from the headspace to the fibre. The following section has a lesser slope. The lesser slope is associated with analytes partitioning from the aqueous matrix to the headspace and from there to the

fibre. It is limited by the rate at which analytes partition from the aqueous phase to the headspace and therefore is not as pronounced as the initial slope. True equilibrium extraction time has been reached when this second slope levels off completely indicating equilibrium between aqueous sample, headspace and fibre. For all three compounds under investigation the equilibrium extraction time appears to have been reached within five minutes.

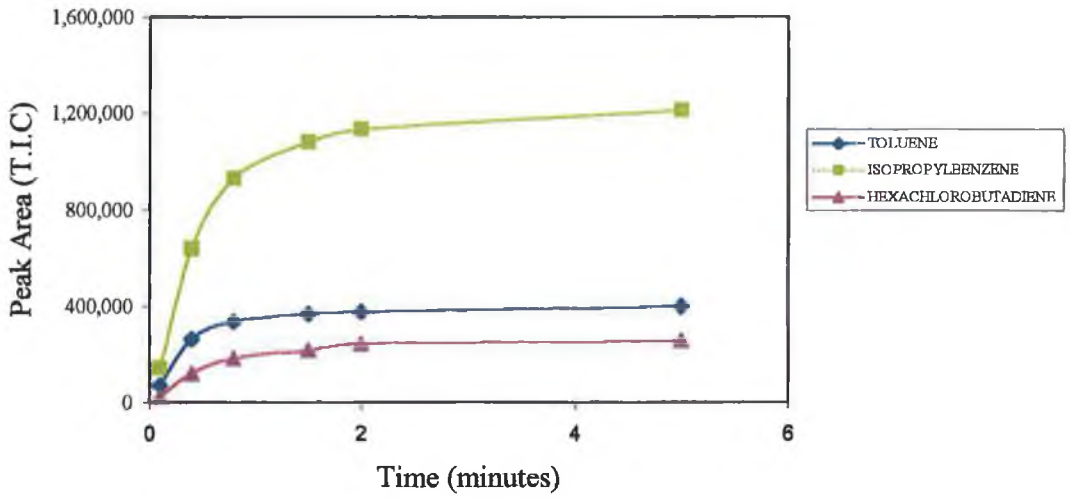

Figure 2.2 Extraction profile for toluene, isopropylbenzene and hexchlorobutadiene obtained using the 100μm PDMS fibre

Toluene, the most volatile of the three compounds, reaches its equilibrium extraction time first, as can be seen by levelling of the extraction profile after approximately 1.5 min. Hexachlorobutadiene and isopropylbenzene take longer to reach equilibrium as illustrated by the continuous slope to five minutes.

The profiles shown above demonstrate the importance of carefully timing the extraction time when an extraction time other than the equilibrium extraction time is being used. As can be seen in **Figure 2.2** extraction of isopropylbenzene for 30s

would yield a significantly different result from an extraction carried out for 40 seconds.

The use of a SPME fibre with a thinner coating causes changes in the extraction profile. As expected, equilibrium extraction times are shorter since the coating thickness through which the analyte must diffuse is less. **Figure 2.3** shows that in the case of isopropylbenzene equilibrium extraction times have been reduced from five minutes to one minute. For this application the GC analysis time was much longer than the extraction time (30 min vs 5 min). There is therefore no advantage to using the thinner coating as completion of extraction of one sample can be timed to coincide with the end of the GC analysis of the previous sample. In this case the GC run time determines the sample turnaround time.

For applications such as the analysis of PAHs, phthalates and other semi-volatiles the extraction time often can determine the sample turnaround time. In these cases the reduced extraction times afforded by the thinner coating is certainly advantageous as equilibrium extraction times can be reduced to less than the GC run time resulting in significant reductions on the overall analysis time.

In addition comparison of peak areas in **Figures 2.2 and 2.3** show that for the analysis of VOCs in water the use of a thinner coating is actually disadvantageous as limits of detection suffer.

The amount of analyte extracted by a SPME fibre can be described by the equation

$$n = KV_sC_{aq} \qquad 2.1$$

where n = amount of analyte extracted,

K = the distribution constant,

V_s = the volume of the stationary phase fibre coating,

C_{aq} = the analyte concentration in the aqueous phase.

From this equation it can be seen that as V_s decreases the amount of analyte extracted also decreases.

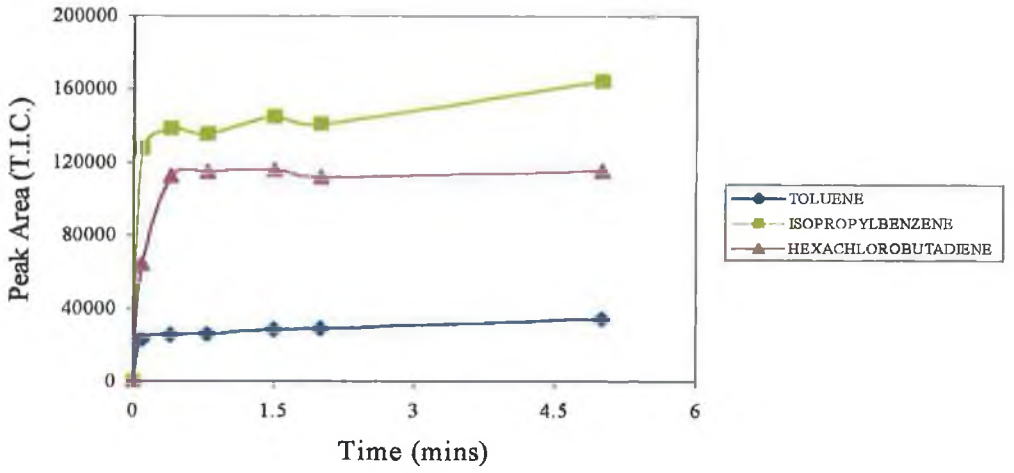

Figure 2.3 Extraction profile for toluene, isopropylbenzene, and hexachlorobutadiene using the 7μm PDMS fibre

2.3.3 Determination of the optimum position of the fibre in the injector

Method precision can be improved by examining the position of the fibre in the injector insert. Different injector ports have different temperature profiles. Generally the centre of the injector is the hottest part but this can vary from injector to injector. It is important that the fibre is placed in the same position in the injector for each injection, otherwise the amount desorbed from run to run will vary. In order to optimise the desorption conditions it is necessary to determine the correct depth for fibre exposure. A 100 μm PDMS fibre was used to extract the headspace above a VOC standard for five minutes followed by desorption for two minutes at 250°C. Figure 2.3 shows the results obtained.

As the fibre is extended further into the injector, the peak area increases. This is due to more complete thermal desorption of the analyte at the higher temperature experienced by the fibre deeper in the injector. It is expected that the response would decrease if the fibre was extended further into the injector, since the injector temperature is highest mid-way along the liner (Injector Temperature Profile **Figure**

1.6). However since the fibre assembly is such that it does not allow the fibre to be placed any deeper into the injector, it was not possible to investigate the response at depths greater than 4.5 cm. Hence the optimum fibre position in the injector was determined to be at the maximum allowable fibre extension.

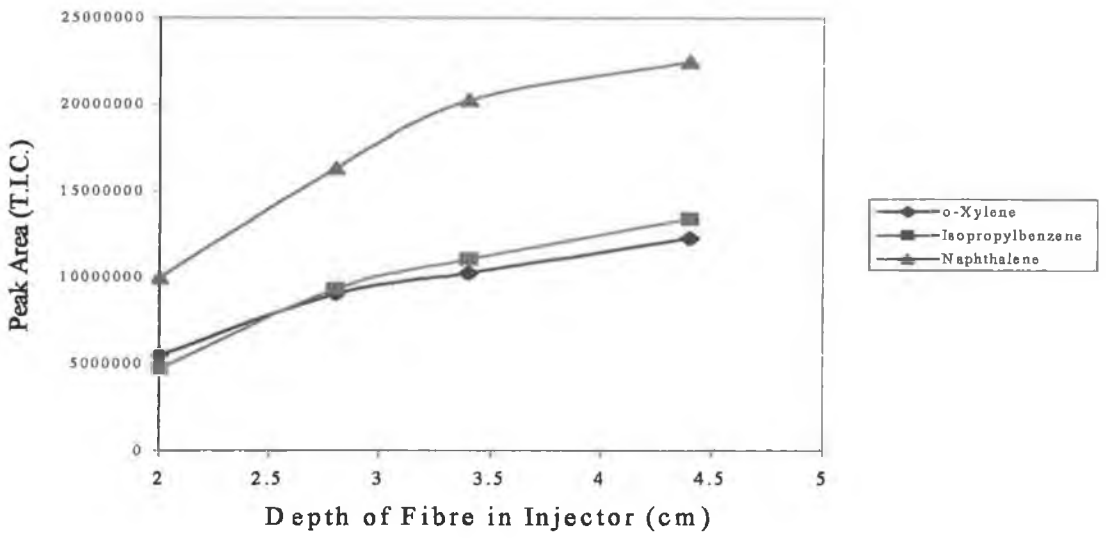

Figure 2.4: Graph showing variation in response with position of SPME fibre in injector port

2.3.4 Selection of fibre coating

A typical mass chromatogram obtained by purge and trap, for the sixty compounds analysed under EPA method 524.2 is shown in **Figure 2.5**. A 10 ppb standard was analysed. These compounds elute over twenty-eight minutes and range in volatility from dichlorofluoromethane (boiling point –29.8 °C) to naphthalene (boiling point 218 °C). The early eluting compounds although very volatile are clearly represented by quite sharp peaks.

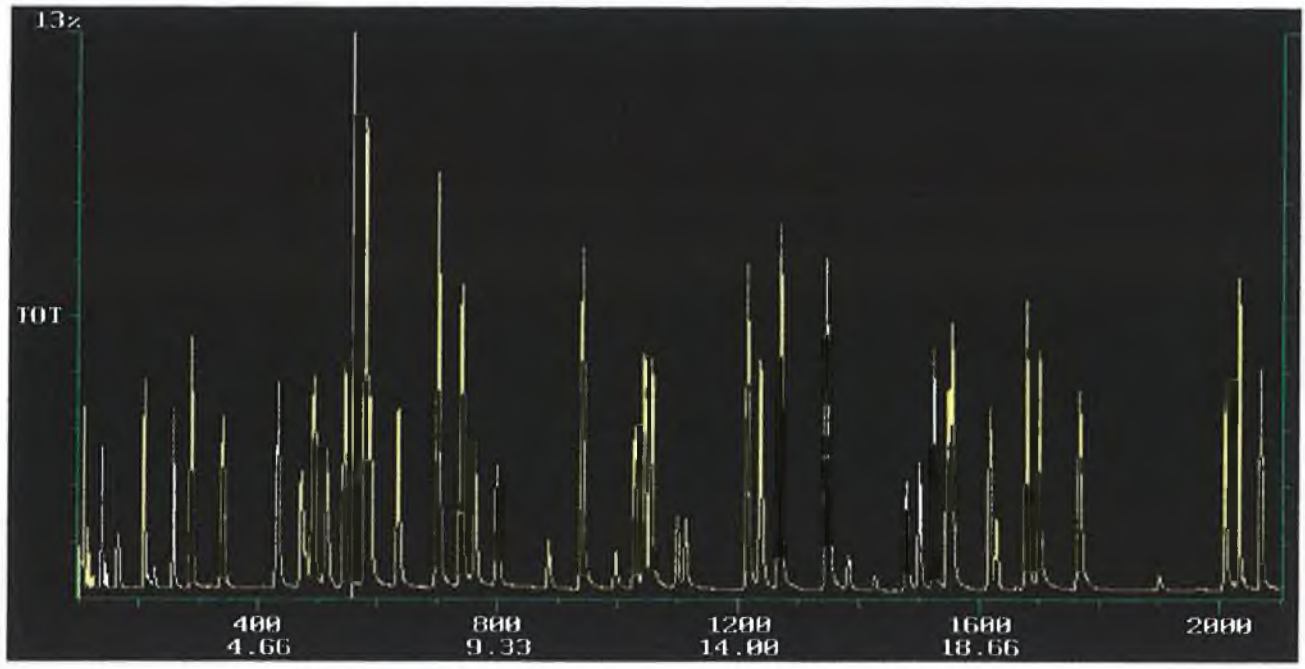

Figure 2.5: Chromatogram of the EPA 524.4 method analytes obtained using Purge and Trap GC-MS

Upon switching to SPME using a 100 μm PDMS fibre a striking difference in the pattern is observed. The intensity of the peaks representing the early eluting compounds dramatically decreases indicating that small volatile analytes are not efficiently extracted (**Figure 2.6**).

The theory and modelling of SPME was initially based on liquid-liquid and gas-liquid adsorption mechanisms. The amount of a particular analyte extracted depends on its retention in the phase coating. No physical bonding actually occurs. The mechanism is dependent upon diffusion in and out of the phase coating. Small analytes which diffuse rapidly are not well retained by the original liquid phases. For this reason the 100 μm PDMS fibre is not suitable for small volatile analytes.

In order to address this problem a new SPME fibre was developed by Supelco, a large chromatography supplies company. The coating on this fibre is a blend of a porous solid material (Carboxen) and a liquid phase (PDMS), the resulting fibre coating being primarily solid. The advantage of these coatings is that they more strongly

retain smaller analytes by physically trapping the analytes in the pores of the materials. Small analytes which rapidly diffuse out of a liquid coating should theoretically be better retained using this fibre.

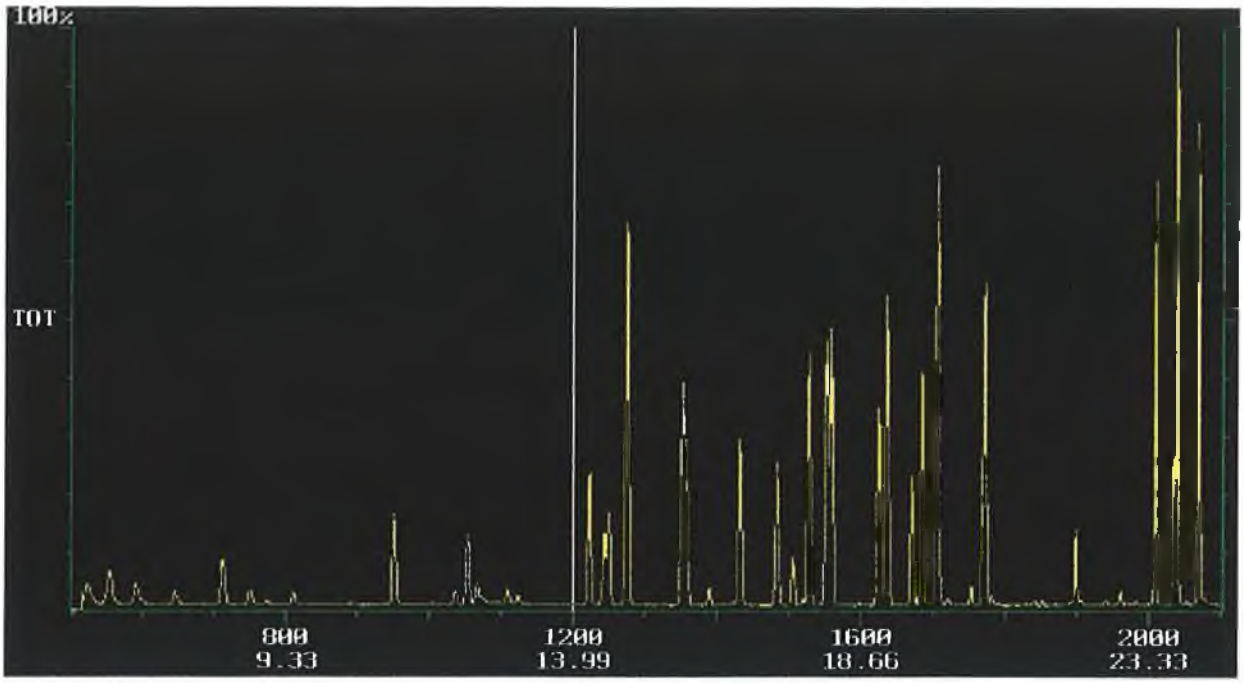

Figure 2.6: Chromatogram of the EPA 524.4 method analytes obtained using SPME-GC-MS (100μM PDMS fibre,10ppb)

Extraction of the same 10ppb standard under the same conditions using the Carboxen/PDMS fibre showed this to be the case as shown in **Figure 2.7**. It can be clearly seen that the Carboxen / PDMS fibre is better suited for extraction of the more volatile analytes. The early eluting compounds increase in intensity relative to the later eluting less volatile components.

Shirey *et al.* have demonstrated that this fibre can be used to extract all 60 VOCs in method 524 down to concentration levels of 1ppb[20]. It was claimed that the analytes' response is strong enough to potentially enable these compounds to be analysed at parts-per-trillion levels. Although a significant improvement in extraction of the more volatile components of the mix was observed when the carboxen / PDMS fibre was used, it was found that purge and trap still performed better than solid phase

microextraction with respect to these compounds. It is also evident by comparing **Figures 2.4 and 2.6** that peak shape of the early eluting compounds is poorer when extracted by SPME. Again this is due to volatility. Purge and trap consists of a cryofocusing step which focuses the analytes into a narrow band before they are flash heated and swept onto the column. SPME does not consist of this step and consequently analyte molecules reach the head of the column in a more diffuse band.

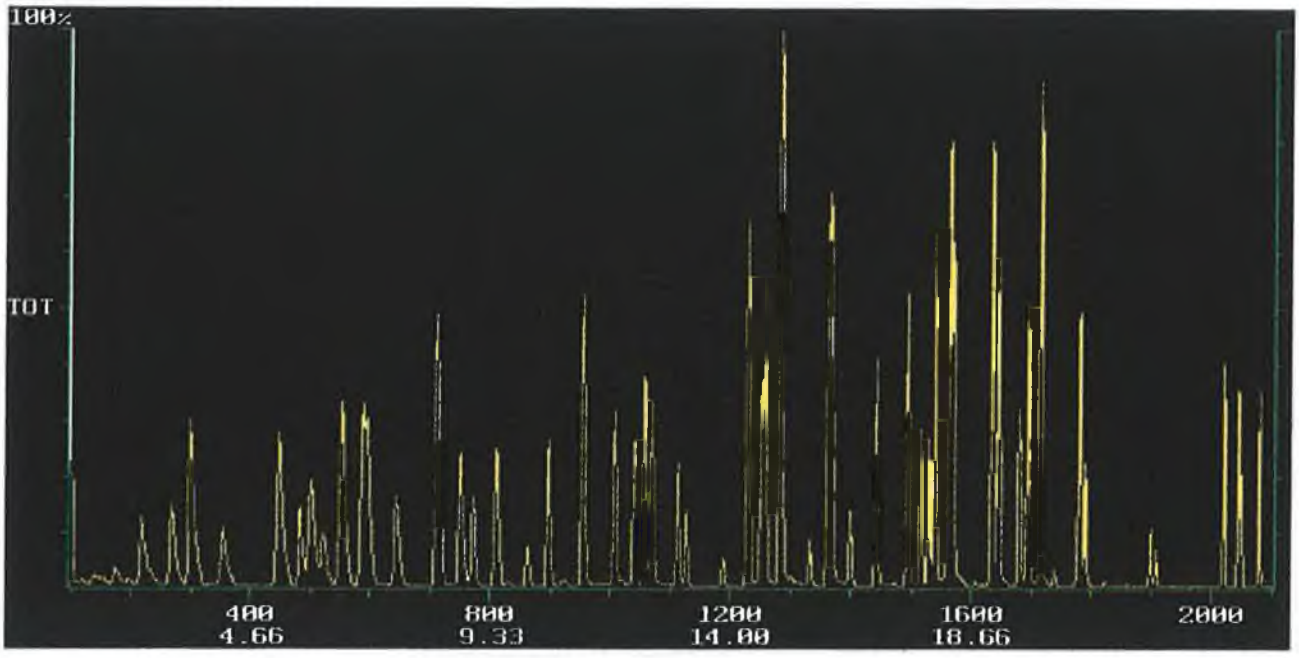

Figure 2.7: Chromatogram of the EPA 524.4 method analytes obtained using SPME GC-MS (75µm Carboxen / PDMS fibre, 10ppb standard)

2.3.5 Optimisation of desorption temperature

The optimum desorption temperature was determined by increasing the temperature of the injector until no carryover was observed on the fibre. The desorption temperature is determined by the compounds with the lowest volatility in the mix, in this case naphthalene and hexachlorobutadiene. Following desorption of the fibre, the now "blank" fibre is re-desorbed. If either naphthalene or hexachlorobutadiene now appear in the chromatogram it can be concluded that the initial desorption temperature was not sufficient. The desorption temperature is increased and the process repeated

until no carryover is observed. For the compounds under investigation the optimum desorption temperatures for the 100 μm PDMS fibre and 75 μm Carboxen / PDMS fibre were determined to be 220 °C and 250 °C respectively. The higher desorption temperature required by the Carboxen / PDMS fibre reflects the greater energy required to release the extracted analytes. This is due to physical trapping of the analytes in the porous Carboxen material.

Another approach to optimising the desorption temperature is to use the maximum allowable coating temperature as the injector temperature and then to adjust the desorption temperature. This approach was not adopted as it can result in a higher amount of bleed from both the fibre and septum.

2.3.6 Repeatability of the method

To ensure repeatability of analysis when using SPME a number of parameters have to be optimised. As was discussed in **Section 2.2.1** extraction times should be carefully controlled, particularly when an extraction time less than the equilibrium extraction time is being used, as the amount of analyte adsorbed onto the fibre can vary significantly over a short time period. Desorption times must also be carefully monitored to prevent variations in the amount of analyte desorbed onto the column. If possible, an automated system should be used to reduce variation between extractions as RSD values can be many times higher for some compounds when manual SPME is used instead automated SPME.

The dimensions of the vial used to hold the sample can also affect repeatability. Long thin vials can be difficult to stir which has implications for SPME as analyte transfer to the fibre will vary from run to run. When carrying out headspace SPME, vials with the minimum headspace required to accommodate the fibre comfortably should be used. They should have a uniform diameter and glass thickness so that variations in headspace volume are minimised.

Seven replicate extractions were carried out on a 10 ppb VOC standard to assess the repeatability of the method. Extractions were carried out for 5 min on the headspace above a 0.8 ml aliquot of the standard mix in a 2 ml autosampler vial. The fibre was desorbed for 2 min at 220 °C while the injector was operated in the splitless mode for 0.7 min. The results are shown in **Table 2.1.**

Table 2.1: Data for Replicate Extractions.*Outlier - not included in RSD calculation

Run Number	Peak Area - Total Ion Count (T.I.C.)			
	Benzene	o-Xylene	Isopropylbenzene	Naphthalene
1	**100,784**	**1,439,929**	**1,122,960**	**278,828**
2	**102,376**	**1,485,830**	**1,174,683**	**286,521**
3	**101,247**	**1,412,854**	**1,145,299**	**278,521**
4	**101,301**	**1,453,096**	**1,188,199**	**306,457**
5	**102,015**	**1,478,901**	**1,666,290***	**283,419**
6	**100,726**	**1,436,436**	**1,172,868**	**300,389**
7	**104,697**	**1,471,493**	**1,160,366**	**304,578**
RSD (%)	**1.3**	**1.8**	**2.0**	**4.1**

The above data shows that with careful control of parameters good repeatability can be obtained for extraction of VOCs from aqueous matrices. Previous assessments showed a variety of parameters affected repeatability. Extracting smaller headspace volumes, careful control of extraction time and monitoring of fibre position in the injector all had positive effects on repeatability. Automated extractions using smaller headspace volumes were found to be up to 15 times better than a manual extraction using a larger headspace.

2.3.7 Limit of detection

Table 2.2 shows limit of detection data for both SPME and purge and trap methods. A standard was prepared at 1 µg/l and consistently produced a signal to noise ratio of greater than 5 for those compounds that were detected. Current regulatory requirements do not necessitate lower limits of detection and so further method

development to achieve lower detection limits was not undertaken. The application of salting out techniques would result in improved detection limits for some of these compounds, particularly the less volatile compounds. However, preliminary investigations using salting out suggested that for the early eluting compounds, improvements in detection limits could not be achieved. With the exception of five of the sixty compounds on the list, it was found that SPME can match the limits of detection attained by purge and trap. Shirey *et al.*[25] obtained lower detection limits but work by Young *et al.* also found that SPME detection limits could not match purge and trap detection limits for the more volatile components.

2.3.8 Analyte losses from the fibre

Analyte losses from the 100 μm PDMS fibre in the time between completion of extraction and initiation of injection were investigated. 0.8 ml of a 20 ppb standard was extracted in 2ml vials for 5 min. The response obtained by varying the length of time between extraction and desorption for duplicate analyses are shown in **Fig.2.8.**

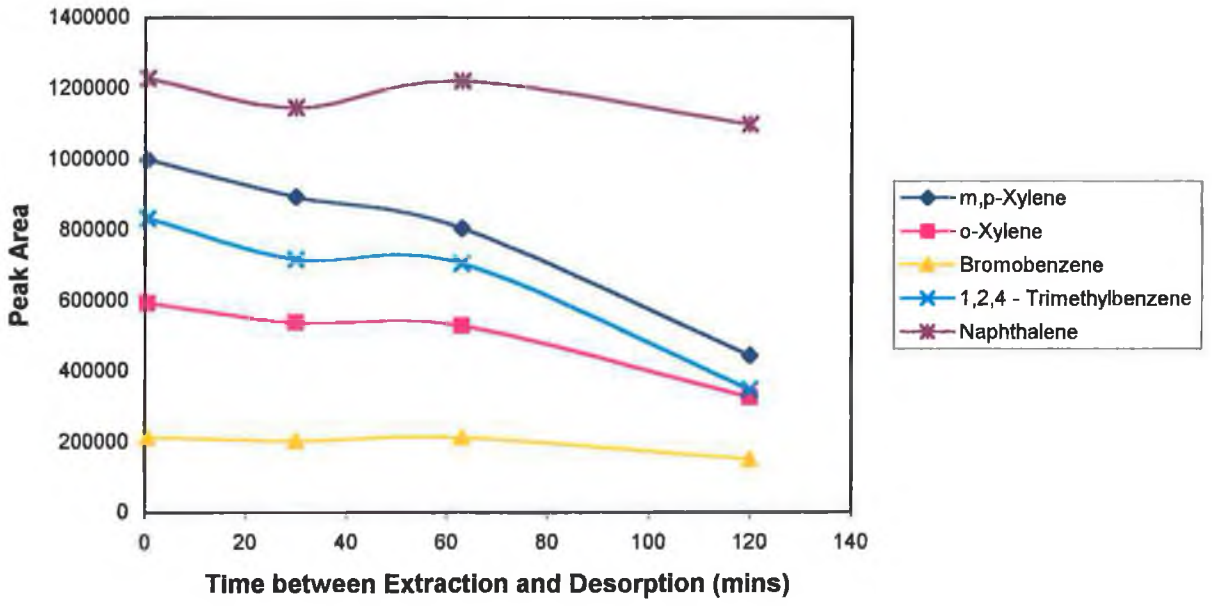

Figure 2.8: Graph showing analyte loss from the 100μm PDMS fibre over a 2 hour time period

Table 2.2: Limits of Detection and Correlation Coefficients of Calibration Curves for SPME and Purge and Trap analysis using 100µm PDMS fibre. *Using 75µm Carboxen/PDMS fibre (N.D.= Not detected)

Compound	SPME Limit of Detection	Purge and Trap Limit of Detection	Average Calibration Curve Correlation Coefficient (SPME) n=5	Average Calibration Curve Correlation Coefficient (Purge and Trap) n=5	Compound	SPME Limit of Detection	Purge and Trap Limit of Detection	Average Calibration Curve Correlation Coefficient (SPME) n=5	Average Calibration Curve Correlation Coefficient (Purge and Trap) n=5
Dichlorodifluoromethane*	N.D.	1 µg / l	N.D.	0.997	Chlorobenzene	1 µg / l	1 µg / l	0.999	1.000
Chloromethane*	N.D.	1 µg / l	N.D.	0.998	1,1,1,2-Tetrachloroethane	1 µg / l	1 µg / l	0.994	1.000
Vinyl Chloride*	N.D.	1 µg / l	N.D.	1.000	Ethylbenzene	1 µg / l	1 µg / l	1.000	0.999
Bromomethane*	N.D.	N.D.	N.D.	N.D.	m-Xylene	1 µg / l	1 µg / l	1.000	1.000
Chloroethane*	N.D.	N.D.	N.D.	N.D.	p-Xylene	1 µg / l	1 µg / l	1.000	1.000
Trichlorofluoromethane*	1 µg / l	1 µg / l	0.996	1.000	o-Xylene	1 µg / l	1 µg / l	0.992	1.000
1,1-Dichloroethene*	1 µg / l	1 µg / l	0.998	0.996	Styrene	1 µg / l	1 µg / l	0.990	0.998
Methylene Chloride*	1 µg / l	1 µg / l	0.995	1.000	Isopropylbenzene	1 µg / l	1 µg / l	0.999	1.000
Trans- 1,2 – Dichloroethene*	1 µg / l	1 µg / l	1.000	1.000	Bromoform	1 µg / l	1 µg / l	0.999	0.999
1,1 – Dichloroethane*	1 µg / l	1 µg / l	1.000	0.999	1,1,2,2-Tetrachloroethane	1 µg / l	1 µg / l	0.995	0.999
cis – 1,2 – Dichloroethene*	1 µg / l	1 µg / l	0.999	0.999	1,2,3-Trichloropropane	1 µg / l	1 µg / l	0.999	0.999
2,2 – Dichloropropane*	1 µg / l	1 µg / l	1.000	1.000	n-Propylbenzene	1 µg / l	1 µg / l	0.997	0.999
Bromochloromethane	1 µg / l	1 µg / l	0.998	1.000	Bromobenzene	1 µg / l	1 µg / l	0.989	0.998
Chloroform	1 µg / l	1 µg / l	0.999	1.000	1,3,5-Trimethylbenzene	1 µg / l	1 µg / l	0.990	1.000
1,1,1 – Trichloroethane	1 µg / l	1 µg / l	1.000	1.000	2-Chlorotoluene	1 µg / l	1 µg / l	0.999	0.998
1,2 – Dichloroethane	1 µg / l	1 µg / l	0.998	0.986	4-Chlorotoluene	1 µg / l	1 µg / l	0.999	0.999
1,1 – Dichloropropene	1 µg / l	1 µg / l	0.999	0.999	Tert-Butylbenzene	1 µg / l	1 µg / l	1.000	0.999
Benzene	1 µg / l	1 µg / l	1.000	1.000	1,2,4-Trimethylbenzene	1 µg / l	1 µg / l	0.999	0.999
Carbon tetrachloride	1 µg / l	1 µg / l	0.997	1.000	Sec-butylbenzene	1 µg / l	1 µg / l	0.998	0.998
1,2 – Dichloropropane	1 µg / l	1 µg / l	0.990	0.995	p-isopropyltoluene	1 µg / l	1 µg / l	0.991	0.998
Trichloroethene	1 µg / l	1 µg / l	0.999	1.000	1,3-Dichlorobenzene	1 µg / l	1 µg / l	1.000	1.000
Dibromomethane	1 µg / l	1 µg / l	0.997	1.000	1,4-Dichlorobenzene	1 µg / l	1 µg / l	0.998	0.998
Bromodichloromethane	1 µg / l	1 µg / l	0.990	0.999	n-butylbenzene	1 µg / l	1 µg / l	1.000	1.000
Trans – 1,3 – Dichloropropene	1 µg / l	1 µg / l	0.998	0.999	1,2-Dichlorobenzene	1 µg / l	1 µg / l	0.998	0.999
Cis – 1,3 – Dichloropropene	1 µg / l	1 µg / l	0.999	1.000	1,2-Dibromo-3-chloropropane	1 µg / l	1 µg / l	0.998	0.995
Toluene	1 µg / l	1 µg / l	1.000	1.000	1,2,4-Trichlorobenzene	1 µg / l	1 µg / l	0.999	0.999
1,1,2 – Trichloroethane	1 µg / l	1 µg / l	0.990	0.999	Hexachlorobutadiene	1 µg / l	1 µg / l	0.998	1.000
1,3 – Dichloropropane	1 µg / l	1 µg / l	0.989	0.999	Naphthalene	1 µg / l	1 µg / l	1.000	1.000
Dibromochloromethane	1 µg / l	1 µg / l	0.999	0.997	1,2,3-Trichlorobenzene	1 µg / l	1 µg / l	0.998	1.000
1,2 – Dibromomethane	1 µg / l	1 µg / l	0.995	0.999	Fluorobenzene	1 µg / l	1 µg / l	Used as an internal standard	
Tetrachloroethane	1 µg / l	1 µg / l	0.998	0.999	1,2-Dichlorobenzene-d_4	1 µg / l	1 µg / l	Used as an internal standard	

These results show that in general, analyte losses from the 100 μm PDMS fibre are not significant over time periods of less than one hour at room temperature (19 °C) but ideally the time between extraction and desorption should be kept to a minimum. The degree to which an analyte is lost from a fibre is dependent on the fibre, temperature of the surroundings, air flow over the fibre, the time between sampling and analysis, and the analyte.

More volatile compounds are lost to a greater extent from the fibre than less volatile ones. This can be seen when naphthalene loss is compared to m,p-Xylene loss. An 11% decrease in response is noted for napthalene over 2 hours while the more volatile m,p-Xylene decrease in response was far greater at 66%.

K_{fg}, the fibre coating / gas phase distribution constant can be defined as

$$K_{fg} = \frac{\text{Analyte concentration in the fibre}}{\text{Analyte concentration in gas phase surrounding fibre}}$$

An analyte with a large K_{fg} is not as readily lost from the fibre as one with a smaller K_{fg}. The larger the analyte's K_{fg} the less will be the analyte loss. Simple precautions such as storage of the fibre in a refrigerator between sampling and analysis and capping of the fibre with a septum greatly reduce analyte loss.

An investigation of analyte loss from the fibre is very important when the fibre is being used for on site sampling, as the time between sampling and analysis can be significant. Capping the fibre with an injector septum effectively seals the fibre in the needle and therefore reduces analyte losses. In those situations where the time between sampling and analysis is quite long, particularly for compounds with high volatility, conventional sample storage techniques should be used such as storage of water samples in sealed vials or air samples in tedlar bags. Micro-extraction of the sample can then be performed in the laboratory at the time of analysis.

2.3.8 Analysis of water samples

The SPME method developed for the analysis of VOCs in aqueous standards was applied to the analysis of three different types of samples, groundwater, industrial effluent and HPLC grade water. Analysis was carried out to ensure that these samples did not contain the volatile organic compounds under investigation. These samples were then spiked with 1,1,1-trichloroethane, benzene, toluene, m,p-Xylene, o-Xylene, 1,3,5-trimethylbenzene, naphthalene and hexachlorabutadiene each at a concentration of 8.2 μg/l.

Table 2.3: Concentrations of VOCs / recoveries obtained using 100μm PDMS fibre, headspace sampling for 5 minutes, 2 minute desorption at 220°C

Compound	Concentration (μg/L) / Recovery(%) n=3		
	HPLC Water	Groundwater	Industrial Wastewater
1,1,1 trichloroethane	7.9 / 96%	7.8 / 95%	7.8 / 95%
Benzene	8.5 / 104%	8.0 / 98%	8.3 101%
Toluene	8.6 / 104%	7.9 / 96%	7.6 / 93%
m,p – Xylene	8.0 / 98%	7.0 / 85%	7.4 / 90%
o-Xylene	7.8 / 95%	7.2 / 88%	7.6 / 93%
1,3,5-trimethylbenzene	7.5 / 91%	7.0 / 85%	6.9 / 84%
Naphthalene	8.1 / 98%	7.9 / 96%	7.0 / 85%
Hexachlorabutadiene	8.1 / 98%	7.9 / 96%	7.2 / 88%

Table 2.4: Concentrations of VOCs / recoveries obtained using Purge and Trap GC-MS

Compound	Concentration (μg/L) / Recovery(%) n=3		
	HPLC Water	Groundwater	Industrial Wastewater
1,1,1 trichloroethane	8.0 / 98%	7.9 / 96%	8.0 / 98%
Benzene	8.4 / 102%	8.0 / 98%	8.0 / 98%
Toluene	8.2 / 100%	8.0 / 98%	7.8 / 95%
m,p – Xylene	8.2 / 100%	7.7 / 94%	7.7 / 94%
o-Xylene	8.1 / 96%	8.1 / 99%	7.8 / 95%
1,3,5-trimethylbenzene	7.9 / 109%	7.5 / 91%	7.4 / 90%
Naphthalene	8.9 / 106%	7.6 / 93%	7.3 / 89%
Hexachlorabutadiene	8.7 / 79%	7.1 / 87%	7.6 / 93%

Tables 2.3 and 2.4 show VOC concentrations calculated using solid phase microextraction and purge and trap based on three replicate measurements. Recoveries from HPLC water are higher than those from groundwater or industrial wastewater. The most likely reason for this is that HPLC water contains none of the particulate organic matter associated with the other two sample types. As a result no analyte losses due to particulate adsorption is observed.

Groundwater recoveries and industrial wastewater recoveries are slightly higher for the purge and trap method than for SPME. Purge and trap is an exhaustive extraction technique, i.e. helium is sparged through the sample until all analyte is stripped from the matrix. Conversely, headspace SPME as used is an equilibrium extraction technique and is not capable to the same extent as purge and trap of extracting those VOCs which are only lightly adsorbed to particulate matter. Consequently, recoveries are lower.

2.3.8 Monitoring of drinking water for trihalomethanes to EU directive 98/33EEC using SPME

Water-borne diseases have dramatically decreased since chlorination was introduced to disinfect drinking water. Trihalomethanes, the byproducts of chlorination, have caused concern due to their association with bladder and colon cancer. Their regulation and monitoring have therefore become an important issue.

Purge and trap - gas chromatography is the method adopted by the US EPA for analysis of trihalomethanes. An adaptation of the SPME method described for the analysis of those VOCs analysed for under EPA method 524, of which the trihalomethanes are members, provides an alternative simple and rapid method for the analysis of these halogenated compounds.

Three samples of commercially available mineral water and four samples of drinking water from local authority water supplies were analysed for the trihalomethanes chloroform, bromodichloromethane, chlorodibromomethane and bromoform. A 100 μm PDMS fibre was used to extract the headspace above the sample for 2 min at

ambient temperature. The extracted analytes were then desorbed for 0.7 min at 220 °C. Fibre blanks carried out after desorption as expected showed no carryover as the highest boiling point of the analytes (Tribromomethane at 149.6°C) is well below the desorption temperature. No column cryofocussing was required. The run time was 20 minutes. 0.5 μg/l of each trihalomethane could easily be detected and the use of an ECD detector, which is selective for halogenated compounds would most likely result in even lower limits of detection if required. The EU directive recommends that total trihalomethanes should not exceed 100 μg/l. The results of the analysis based on duplicate measurments are shown in **Table 2.5.**

Table 2.5: Concentration of trihalomethanes in various water samples determined using SPME-GC-MS (N.D. = Not detected)

Sample	Concentration (μg/l)				
	Chloroform	Bromodichloromethane	Chlorodibromomethane	Bromoform	Total Trihalomethanes
Mineral Water 1	N.D.	N.D.	N.D.	N.D.	N.D.
Mineral Water 2	N.D.	N.D.	N.D.	N.D.	N.D.
Mineral Water 3	16	2	N.D.	N.D.	18
Local Authority 1	34	12	7	N.D.	53
Local Authority 2	63	15	4	2	84
Local Authority 3	48	9	8	3	68
Local Authority 4	45	12	6	N.D.	63

Trihalomethanes are formed in water from the interaction of organic matter with chlorine, which is used to kill any harmful bacteria that may be present in the water. Mineral water is not treated with chlorine as it is assumed not to be contaminated with harmful bacteria. The results show that one of the companies marketing mineral water must be treating the water before bottling as trihalomethane by-products are present.

Analysis of a number of water samples from different local authority water supplies showed that trihalomethanes were clearly present. The trihalomethane concentration in some cases approached the directive value but in no case was it exceeded. Should this directive become law, SPME-GC-MS would prove to be an invaluable tool to the regulatory body charged with policing trihlomethane concentrations in drinking water.

The automated systems available are simple in design and have lower operating and service costs than automated purge and trap systems. Sample throughput is determined by gas chromatography run times and detection limits in low µg/l levels are easily attainable.

2.4 Conclusion

SPME can be applied to the analysis of a range of volatile organic compounds. The 100 µm PDMS fibre is suitable for the extraction of 46 of the 58 volatile organic compounds on US EPA method 524.2 list when using headspace sampling at room temperature followed by desorption at 220°C. An additional 7 of the more volatile compounds could also be extracted using the 75 µm carboxen / PDMS fibre, followed by desorption at 250 °C. The main advantage this technique has over the more widely used purge and trap analysis is its simplicity – it does not require the complex instrumentation associated with purge and trap. It is rapid, easy to operate and automate, has minimum maintenance and does not require expensive high-purity purge gases. Purge and trap analysis of water samples which contain a significant amount of particulates and / or bacteria have proven to be very problematic in the past due to blockages and growth of bacteria within the instrument. SPME can overcome this problem as headspace SPME sampling isolates the sample from all instrumentation. As a replacement for purge and trap, however, SPME has one serious limitation – it can not extract the very light VOCs, dichlorodifluoromethane, chloromethane, vinyl chloride, bromomethane and chloroethane, at low µg/l levels. Column cryofocussing, the use of internally cooled fibres and further development of new fibre coatings in the future may overcome this problem.

As the public become increasingly aware of the quality of their drinking water, mounting pressure is being placed on governments, regulatory bodies and local authorities to provide a clean and safe water supply. A recent EU directive, calling for limitation of the total trihalomethane concentration in water to less than 100 µg/l, is putting increasing pressure on local authorities to monitor their water for chlorination by-products. SPME-GC has been shown to provide a quicker, simpler alternative to purge and trap - GC for assessing trihalomethane concentrations.

2.5 References

[1] P. Kuran, L. Sojak, J. Chromatogr. A, **733** (1996) 119.

[2] R. Gobel, R. Krska, R. Kellner, R.W. Seitz and S.A. Tomellini, Appl. Spectros., **48** (1994) 678.

[3] M. Gotah, Y. Sekitani, H. Kobayashi, K. Ognio and T. Hobra, Bull. Environ. Contam. Toxicol., **49** (1992) 186.

[4] M.D. Reuber, Environ. Health Perspect., **31** (1979) 171.

[5] J.P. Velema, J. Environ. Sci. Health, **5** (1987) 1.

[6] M. Mohnke and J. Buijten, Chromatographia, **37** (1993) 51.

[7] E.C. Vigliani, Ann. N.Y. Acad. Sci., **271** (1976) 143.

[8] S.C. Rastogi, Bull. Environ. Contam. Toxicol., **50** (1993) 811.

[9] R.A. Rinski, A.B. Smith, R. Hornung, T.G. Filoon, R.J. Young, A.M. Okun and P.J. Londrigan, N. Eng. J. Med, **316** (1987) 1044.

[10] R. McCallum, P. Pendleton, R. Schumann and M.U. Trinh, Analyst, **123** (1998) 2155.

[11] M. Guidotti, R. Giovinazzo, O. Cedrone, M. Vitali, Environment International, **26** (2000) 23.

[12] K.J. James and M.A. Stack, Fresnius J. Anal. Chem., **358** (1997) 833.

[13] P.T. Kostecki and E.J. Calabrese, Hydrocarbon Contaminated Soils and Groundwater, Lewis, Chelsea, MI (1991).

Chapter Three

Plasticisers in Children's Toys

3.1 INTRODUCTION

Plasticisers are organic compounds added to polyvinyl chloride (PVC) to impart flexibility to its otherwise rigid structure. The majority of plasticisers used are phthalate esters, otherwise known as benzenedicarboxylic acid esters. Other compounds with similar structures such as citrates, adipates and sebacates have also been used. The most commonly used vinyl plasticisers are diethylhexyl phthalate (DEHP) and diisononyl phthalate (DINP). DEHP is most commonly used in the manufacture of vinyl medical devices, while DINP is most commonly used in the manufacture of vinyl children's products, construction materials and other consumer products. Over half the weight of some flexible PVC products can consist of phthalates. Since phthalates are not chemically bound to the PVC polymer itself they readily leach out of PVC products. Up to 1% of the phthalate content of PVC products can be released each year[1]. As a result of their continuous release during the production, use and disposal of PVC products, phthalates are often described as "ubiquitous" or "the most abundant man made environmental pollutants".

Both DINP and DEHP have been shown to cause adverse effects when administered to laboratory animals. As a result, certain groups have expressed concern over the use of these compounds in plastics suggesting that they pose a threat to human health and should be banned. Advocates of their use argue that the mechanisms by which phthalates cause ill effects in laboratory animals may not exist in humans and that the doses to which these animals are exposed are many orders of magnitude greater than those to which humans are exposed.

Concerned groups have campaigned for a ban on the use of phthalates in children's toys and childcare articles arguing that infants are being unnecessarily exposed to these chemicals when they mouth the product. Regulatory bodies have been slow to pose an outright ban on products containing these compounds due to the huge implications on the industries producing these products, in addition to the lack of known safer substitutes. One possible alternative to a total ban which has been suggested is to ban only those products which leach phthalate above a threshold rate.

3.2 CHEMICAL PROPERTIES OF DINP AND DEHP

DINP: The structure of DINP is shown below. Commercially available DINP is not a single compound but a mixture of several different isomers. Furthermore the mixture of isomers varies from manufacturer to manufacturer and any single manufacturer's DINP can also vary from batch to batch. Such variations are unlikely to have any affect on the performance of the phthalate in the plastic but they are quite likely to have an effect on the level of toxicity to organisms[2].

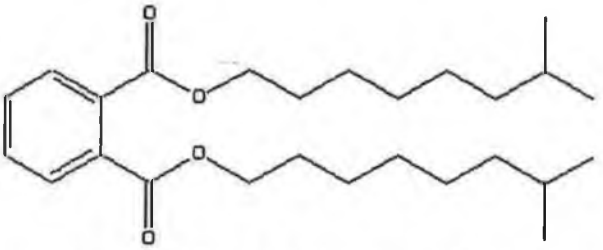

Figure 3.1: The Structure of DINP

The main chemical properties of DINP are outlined in **Table 3.1**.

Chemical Formula	C_{26} H_{42} O_4
Molecular Weight	418.62
Synonyms	1,2-Benzenedicarboxylic acid diisononyl ester; bis(7-methyloctyl) phthalate; di-"isononyl" phthalate
CAS Number	28553-12-0 68515-48-0
Flash Point	>93.3°C
Solubility in Water	>1mg/L @ 21°C according to Exxon manufacturers = 0.224 ± 0.1mg/L in distilled water according to the US EPA
Vapour Pressure	= 7.2 x 10^{-5} @ 25°C analysis by GC
Physical Appearance	Clear, colourless liquid

Table 3.1: Physical and chemical properties of DINP

DEHP: DEHP is similar in structure to DINP and is shown Figure 3.2. DEHP is available as a single isomer. Its chemical properties are summarised in Table 3.2

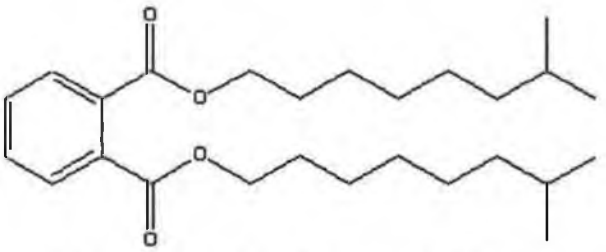

Figure 3.2: The Structure of DEHP

Chemical Formula	$C_{26}4H_{38}O_4$
Molecular Weight	390.56
Synonyms	1,2-Benzenedicarboxylic acid bis(2-ethylhexyl) ester
CAS Number	117-81-7
Flash Point	199°C
Specific Gravity	0.985g/ml
Physical Appearance	A clear viscous liquid with slight odour

Table 3.2: Physical and chemical properties of DEHP

3.3 SOURCES OF HUMAN EXPOSURE TO DINP AND DEHP.

Humans are exposed to DINP, DEHP and other di-alkyl phthalates (DAPs) from a wide variety of sources. Any PVC or plastic-containing product may act as a potential source. Exposure can be by ingestion, inhalation, direct injection or skin contact. Absorption rates vary dramatically among the different exposure pathways and the phthalate can undergo modification during absorption / exposure[3].

Absorption via inhalation is quite an effective ingestion pathway. One of the most common sources of breathable phthalates is the interior of cars due to their high plastic content and the high temperature which can be reached. Food, particularly fatty food that has been in contact with vinyl products, is the main source of ingestible

phthalates. For babies and young children, in addition to food sources, the mouthing of plastic toys and childcare articles is also another significant source. Phthalates may be inadvertently injected as a result of medical treatments such as blood transfusions where the phthalate may leach from the PVC bag containing the blood. Skin absorption of phthalates is not thought to be a major source. In a test of skin penetration, where a phthalate solution was painted onto the skin of a laboratory rat, 86% of the chemical stayed on the skin and was not absorbed, even after seven days[4].

3.4 TOXICITY TESTING ON ANIMALS

DINP and DEHP have been tested on a variety of animals including rats, mice, rabbits, monkeys, dogs and cats. Based on standard toxicology testing DEHP shows very low acute toxicity, that is, low toxicity from a single high dose. The "benchmark" measure of toxicity is called the LD_{50}, the dose at which 50% of a test population dies upon exposure to a given substance. LD_{50} values for injection of rats with DEHP are approximately the same as for feeding an equivalent value of caffeine. DINP would also be classed as having low acute toxicity.

Long-term exposure of different laboratory animals to these two phthalates have resulted in the observation of a variety of adverse effects. Toxicity testing of rats and mice has shown that DINP damages both the kidneys and liver. An increase in the size of these organs can occur at levels lower than that at which carcinogenic effects are observed[5]. One physiological process that is used to gauge the cancer causing potential of a chemical is its ability to cause peroxisome proliferation. In peroxisome proliferation studies evidence that certain cell bodies called peroxisomes have developed at abnormally high levels in liver cells or other suspected sites of cancer formation is sought following exposure to the chemical. Both DINP and DEHP can be classed as peroxisome proliferators[5, 6]. DINP has been shown to cause mononuclear cell leukaemia[5] while DEHP causes liver tumours[5] in rats. DEHP has also been known to damage the heart and lungs and although no specific studies on the impact of DINP on these two organs have been carried out they are considered to be potential sites of damage.

Many phthalates are considered to be estrogen mimickers. DINP has shown "extremely weak estrogenic activity" in a recent in-vitro study[7]. DEHP has been

shown to decrease the amount of thyroxine, a thyroid hormone, produced. This is a common property of many compounds capable of promoting tumour growth. Rats fed high doses of DEHP have also been shown to experience reversible damage to the testes, including reduction in testicular size and decreased sperm production[8]. Less damage was seen to the ovaries from similar doses. In a study where DINP was fed to pregnant rats maternal toxicity and developmental effects were seen[9].

3.5 RELEVANCE OF ANIMAL TOXICITY DATA TO HUMANS

Many arguments have been made regarding the applicability of data obtained from animal studies to humans. Human exposures to phthalates are generally many orders of magnitude lower than the doses shown to cause even minor illness in experimental animal subjects. Humans are unlikely to be exposed to comparable levels of the chemical through the same pathway as in the animal studies or for the same period of time. Absorption rates have been shown to vary between species, as does metabolic processing. It is known that metabolic degradation of phthalates requires more steps in rats and mice than it does in primates. It is not clear if it is the phthalate itself or a metabolite that is responsible for toxicity. This could be an important factor since mono(2-ethylhexyl) phthalate, a metabolite of DEHP, is formed at much lower levels within the digestive system of primates and humans than in mice and rats.

The ability of a chemical to cause peroxisome proliferation as mentioned earlier is used as an indication of cancer causing potential. The peroxisome proliferation ability of a chemical varies among animal species. For example, the Syrian hamster is four times less likely to display peroxisome proliferation, when given the same dose of certain peroxisome proliferators, as a mouse or rat. Dogs and rhesus monkeys are even less likely. Huber points out that "the greater sensitivity of the rat to peroxisome proliferators such as DEHP suggest that human risk calculations based exclusively on rat data and dose might lead to an overestimation of the actual threat[4]." Cattley *et al.* argue that the tumours are a secondary effect of peroxisome proliferation. Primates and humans are less sensitive than the mice and rats in which peroxisome proliferation induced tumorigenesis is observed, and, therefore, peroxisome proliferators do not present a cancer hazard in humans[10].

3.6 ACTIONS TAKEN BY REGULATORY BODIES TO THE ISSUE OF PHTHALATE ESTERS IN PLASTIC TOYS

In April 1997 and February 1998, Denmark and Spain, respectively, notified the European Commission, under article 8 of directive 92/59/EEC, of the withdrawal from the market of certain childcare articles and soft PVC toys containing phthalates. In both cases the authorities had concluded that the level of migration of certain phthalates from those specific products, as measured in laboratory tests, did involve a serious risk to children's health.

As a result of these notifications opinion was invited from other member states on this issue. Reaction proved to be varied – certain states called for an outright ban on all products containing these phthalates while other states suggested that products should be subjected to testing and those items leaching phthalates above a certain rate be banned.

An outright ban on the use of phthalates, while definitely eliminating any element of risk to children's health, may not be the wisest step. In the case of a phthalate ban an alternative polymer or plasticiser which meets the same performance requirements and has been demonstrated to be safe is required. Since alternative plasticisers all have similar structures, it is anticipated that their toxicological properties will be similar to those of phthalates. Unfortunately, toxicological data available on the alternatives is often of poor quality, old or non-existent. Alternative polymers to PVC are not available for some applications and those that are available may contain antioxidants, stabilisers and other processing aids that can also migrate, and therefore must be first investigated.

In view of the information available, the questions raised and the divergence of opinions on the subject, the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE) was invited to give its opinion on a number of points. One issue of particular importance was the question of the test method to be followed and the standards or parameters that should be taken into consideration to measure the phthalate migration level. This alternative to an outright ban would identify those products leaching unnecessarily large amounts of phthalates and therefore afford

some degree of protection to the health of the child. The CSTEE recommended in July 1998 that research should be carried out on methods to determine leaching rates. This method should be able to match the leaching rate of 9 ug/hour obtained by the *in vivo* studies carried out by the RIVM, The Netherlands, on reference plastic disks[11]. Results from various studies showed that satisfactory reproducibility of results between laboratories was not attained and the required leaching rate in some cases could not be met. It was decided that continuing work was required in an effort to develop a more robust method.

In the absence of an alternative, and in view of the fact that Member States differed on the adoption measures to deal with the risk in question, the European Commission, on 7th December 1999 adopted a decision banning the placing on the market of childcare articles and toys made of soft PVC, containing specified phthalates intended to be placed in the mouth by children under three. This decision was initially applicable to 8th March 2000 but has since been extended.

Outside of Europe this issue has also resulted in recommendations being made by the Canadian authorities. In November 1998 Health Canada recommended that "parents and caregivers monitor their child's use of small, soft vinyl toys, often placed in playpens or cribs, and to remove these toys if they observe that their child is sucking or chewing on them for prolonged periods of time, on a daily basis."

The US authorities up to early 1999 had not adopted any legislative measures to ban the use of phthalates in children's' toys or childcare articles. The US Consumer Product Safety Commission (CPSC) concluded that based on the best available information about the amount of DINP released from the products tested by them and relying on the exposure information from the Dutch study, few, if any, children are at risk of liver or other organ toxicity from mouthing teethers, rattles, and other PVC toys that contain DINP. However, they did admit that there are a number of significant uncertainties in this assessment including the cancer risk, and further work is necessary to gather better data on which to base the health risk assessment[12].

3.7 APPROACHES TO PHTHALATE MIGRATION TESTING

Methods used to measure the migration rates of phthalate esters from children's toys and childcare articles attempt to simulate what happens during mouthing of the plastic item by the infant. Developing a laboratory based method to accurately determine how much phthalate a child ingests while chewing on a piece of plastic is very challenging. It is complicated by such factors as variations in saliva composition between individuals, the technical feasibility of measuring the amount of phthalate lost to adsorption on the surface of the oral cavity and the fact that young children do not swallow all the saliva they produce. In addition many plastic products are not uniform throughout – certain areas in a sheet of PVC can contain more phthalate than others.

Current methods can be divided into static, dynamic and *in vivo* types. The *in vivo* test method is carried out by allowing adult volunteers chew or suck on plastic items for pre-determined periods of time, collecting the saliva produced and subjecting it to analysis for the leached phthalate. Detractors from this approach point out that adults are more likely to subject the article to greater strain than would a child, in addition to there being significant differences in the composition of adult and infant saliva. Due to ethical considerations these tests have not been carried out using children.

As little information is available on the amount of time infants mouth toys a study was commissioned by The National Institute of Public Health and the Environment (RIVM), The Netherlands to determine average oral contact time. This study took the form of observing the infants on a daily basis and calculating the time per day, during the waking period, a child spends mouthing on an object[11]. Objects were varied and included toys, fingers and other materials available. One scenario could be the abundant availability of PVC toys, leading to the mouthing of PVC toys only, hence all objects were taken into account. The age groups under study were 3-6 months, 6-12 months, 12-18 months and 18-36 months with results shown in **Table 3.3.**

Table 3.3: Standard deviation, minimum, mean and maximum total mouthing time in minutes

	Standard Deviation	Minimum	Mean	Maximum
3-6 months	19.1	14.5	36.9	67.0
6-12 months	44.7	2.4	44.0	171.5
12-18 months	18.2	0	16.4	53.2
18-36 months	9.8	0	9.3	30.9

A human volunteer study to determine release rates of DINP from PVC samples into saliva was also undertaken[11]. Differences in the way of chewing and sucking on toys between children and adults were not considered to introduce a significant error in the exposure estimation. The inter-individual variation in composition of saliva (e.g. pH and protein content) was found not to influence the rate of DINP release. Differences in the composition of saliva of children and adults may have been more significant but as no information on this subject was available to the investigators such differences were ignored. Each individual's saliva was collected over the duration of the study and analysed. The amount of DINP present in each saliva sample was extrapolated back to determine DINP release rates. Allowances were not made for phthalate losses due to adsorption on the surface of the oral cavity; however such losses were thought to be more than offset by the fact that all saliva was collected from the adult volunteers during the mouthing period while in young children a significant portion is lost through dribbling. Although the *in vivo* approach is not suitable for routine analysis, in the light of limited data of this type and its practical nature, this study may provide a more realistic view of phthalate leaching during mouthing.

In contrast to *in vivo* methods, both static and dynamic *in vitro* methods involve placing a test portion of the item in contact with an artificial saliva simulant for a pre-determined time. Real saliva is not used as it is not readily available and its use poses a potential biological hazard to investigators. The test portion used is generally 10 cm^2 in area as observational studies carried out in The Netherlands indicate this to be

the area of item in contact with an infant's mouth at any one time[11]. The artificial simulant is then separated from the test piece, the leached phthalate extracted from the simulant and its concentration determined. In static methods the test piece or saliva is not agitated in any way whereas during dynamic methods an attempt to imitate chewing by employing some physical force to mirror the abrasive effects experienced by a plastic item in the child's mouth is employed.

The migration rate measured depends largely on the method used. Reported migration rates vary from 1μg/10cm^2/hour to 2327μg/10cm^2/hour, with static methods having lower migration rates than dynamic methods[12]. In the absence of a standardised method it is very difficult to ascertain which products pose a greater threat than others. To this end that method which provides the best repeatability and in the opinion of experts most closely mimics what actually occurs during mouthing is currently being sought. The majority of work in the area of phthalate migration testing has been carried out by three main groups

1. The Laboratory of the Government Chemist, United Kingdom
2. The National Institute of Public Health and the Environment (RIVM), The Netherlands and
3. Consumer Product Safety Commission, United States

3.7.1 The Laboratory of the Government Chemist, United Kingdom

The aim of The Laboratory of the Government Chemist (LGC) was to develop a simple, repeatable and correlative laboratory based method which ultimately may be suitable to be adopted as the standard EU test method. The accurate duplication of human mouthing conditions in a laboratory-based method is not possible. In order to make the test method accessible to most laboratories, no custom made or specially designed equipment was used. Instead equipment, which would be commonly found in most well equipped laboratories, was utilised.

The basis of the LGC method involves cutting a disc shaped sample from the test article, placing it in a conical flask along with the saliva simulant and some glass balls

for abrasion. The conical flask is placed in a shaking water bath thermostatted at 37 °C and shaken for a certain time period. The saliva simulant is then decanted, solvent extracted and the extract analysed by GC-MS. During the development of this method a number of factors thought to influence the migration of phthalate plasticisers were examined, taking into account information available from other sources. These factors included sample size and preparation, composition of "saliva simulant" solution, means of mechanical agitation, agitation frequency and contact time[13]. Results obtained did not match with the Dutch *in vivo* migration results so a modification of the method was made to incorporate harsher agitation. Results obtained by LGC using this method were claimed to correlate closely with the *in vivo* 'chew and spit' studies[14]. The next stage in the work carried out by the LGC was to validate the method developed. In addition to in-house method validation, six laboratories took part in an inter-laboratory validation exercise[15]. The method was proposed as the method to be used should children's toys and childcare articles be subjected to a maximum allowed phthalate migration level. It is necessary that in such a case a method which can provide a reliable result is available due to the implications of an inaccurate and imprecise result. As a result of variations in results between laboratories, the method in the format presented was not adopted but cited as a possible means to protecting infants from over exposure to phthalates from plastic toys.

3.7.2 The National Institute of Public Health and the Environment (RIVM), The Netherlands,

In addition to the human volunteer study described in **Section 3.7**, a routine laboratory based method was also developed. This method involved placing a test disc in a head over heels rotator along with the saliva simulant and glass beads to produce a mildly abrasive effect. After a certain time period the simulant is removed, extracted with an organic solvent and analysed by normal phase HPLC with UV detection at 225nm. The effect of a number of parameters on the amount of phthalate extracted was investigated. The aim of the group was to develop a method which would yield results close to those obtained from the *in vivo* studies. The effect of mechanical force was critical in this respect. Best correlation was observed when a

head over heels rotator was used as agitation by other means such as a shaking water bath was found to yield lower results under the conditions used[11]. Following development of the method an inter-laboratory validation exercise was carried out. In common with the LGC's validation exercise the number of participating laboratories was quite limited and quite large variations in results were observed[16].

3.7.3 Consumer Product Safety Commission, United States

Work carried out by The Consumer Product Safety Commission (CPSC) was similar to that carried out by the other two groups. Researchers measured phthalate migration rates from children's products by placing the test product in a stainless steel beaker and immersing it in a saliva simulant at 37°C. A pneumatic piston impacted the product to approximate the effects of a child's biting or chewing. The impaction time was based on data obtained from the RIVM's observation study to determine an infants average mouthing time. The saliva simulant was separated from the sample and phthalates extracted using hexane. The analytical technique used was GC-MS. Total phthalate in the sample was also measured and migration rates did not correlate with the DINP content, manufacturing process or sample thickness[12]. Studies with adult volunteers were conducted to compare the migration rates measured *in vivo* with those measured by the impaction method. Using disks cut from identical toys, the *in vivo* migration rate was, on average, 39.5 times greater than the rate measure by impaction. Therefore a factor of 39.5 was applied to the rates measured by impaction. No data has been published on the validation of this method.

3.8 CONCLUSION

DINP and DEHP, phthalates used in the production of children's toys, have been shown to induce cancer in laboratory animals although the mechanisms by which this occurs, it is argued, may not be relevant to humans. In order to safeguard children's health eight European countries regulated the production and marketing of such products before a temporary Europe wide ban was announced in December 1999. An alternative to a ban which could prevent phthalates being replaced by equally undesirable substitutes is to subject articles to a maximum allowed phthalate

migration level. A number of test methods exist which involve shaking the test item in a saliva simulant followed by measurement of the extracted phthalate.

Two methods presented to the European Commission's Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) were concluded to be unsuitable in their present state for control purposes and were not sufficient to ensure a consistent high level of child health protection. While these methods on their current form were not acceptable, it was considered that changes to the methods might result in a robust method being developed which could be adopted for control purposes in the future.

3.7 References

[1] D.F. Cadogan, " An assessment of the release, occurrence and possible effects of plasticisers in the environment", Brussels, Belgium: European Council for Plastics and Intermediates, 1993.

[2] National Environmental Trust, Washington, US, "A selected annotated bibliography on the toxicity of Diisononyl Phthalate and its migration from children's products", www.envirotrust.com/toyscience.html, 1998.

[3] K. Green, "Phthalates and Human Health: Demystifying the risks of plastic-softening chemicals", www.rppi.org/peg2.html.

[4] W. Huber, Critical Reviews in Toxicology, **26** (1996) 367.

[5] A.W. Lington, M.G. Bird, R.T. Plutnick, W.A. Stubblefield and R.A. Scala, Fund. Appl. Toxicol., **36** (1997) 79.

[6] E.H. Jansen, "Toxicological investigation of di(2-diethylhexyl) ohthalate in rats. The determination of a no effect level" RIVM Report No. 618902007 (December 1992).

[7] C.A. Harris, P. Henttu, M.G. Parker and J.P. Sumpter, Environ. Health Persp., **105** (1992) 802

[8] R.E. Morrissey, M.W. Harris, B.A. Schwetz, Teratogen Carcinogen Mutagen, **9** (1989) 119.

[9] J. Hellwig, H. Freudenberger, R. Jackh, Food Chem. Toxicol., **35** (1997) 501.

[10] R.C. Cattley, J. DeLuca, C. Elcombe, P. Fenner-Crisp, B.G. Lake, D.S. Marsman, T.A. Pastoor, J.A. Popp, D.E. Robinson, B. Schwetz, J. Tugwood and W. Wahli, Regulatory Toxicology and Pharmacology, **27** (1998) 47.

[11] National Institute of Public Health, The Netherlands, "Phthalate release from soft PVC baby toys" RIVM Report No. 613320 002 (September 1998). Published by RIVM, PO Box 1, 3720, Bilthoven, The Netherlands.

[12] U.S. Consumer Product Safety Commission, "The Risk of Chronic Toxicity Assocaited with Exposure to Diisononyl Phthalate in Children's Products - Executive Summary", 1998. Published by U.S. Consumer Product Safety Commission, 4330 East West Highway, Bethesda, MD 20814, United States.

[13] A.O. Earls, C.A. Clay, I.P. Axford, R.P. Scott and J.H. Braybrook, "Laboratory-Based Agitation Methods for the Determination of Phthalate Plasticiser Migration from PVC Toys and Childcare Articles", LGC Technical Report LGC/1998/DTI/009 (1998). Published by LGC, Queen's Road, Teddington, Middlesex TW11 OLY, UK.

[14] I.P. Axford, A.O. Earls, R.P. Scott and J.H. Braybrook, "Laboratory-Based Agitation Methods for the Determination of Phthalate Plasticiser Migration from PVC Toys and Childcare Articles - 2", LGC Technical Report LGC/199/DTI/002 (1999). Published by LGC, Queen's Road, Teddington, Middlesex TW11 OLY, UK.

[15] I.P. Axford, A.O. Earls, R.P. Scott and J.H. Braybrook, "Interlaboratory Validation of Laboratory-Based Agitation Methods for the Determination of Phthalate Plasticiser Migration from PVC Toys and Childcare articles", LGC Technical Report LGC/1999/DTI/004 (1999). Published by LGC, Queen's Road, Teddington, Middlesex TW11 OLY, UK.

[16] TNO Nutrition and Food Research Institute, The Netherlands, "Validation of the method "Determination of Diisononylphthalate in saliva simulant"", TNO report V99.598 (1999). Published by TNO, Ultrechtseweg 48, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

Chapter Four

Determination of leaching rates of DINP from children's toys and childcare articles using SPME/LLE-GC-MS

4.1 INTRODUCTION

The determination of phthalate leaching rates from plastic samples can most realistically be carried out in the laboratory by agitation of the sample in a saliva simulant. The agitation method chosen, leaching time and temperature, and the saliva simulant composition all influence the leaching rate.

The sample was placed in a conical flask with 10 glass balls and 50mls of saliva simulant and shaken for 30 minutes using a shaken water bath. This approach was adapted due to its simple nature and the availability of the equipment required. The shaking of the glass balls against the sample produces a mildly abrasive effect. The temperature chosen was body temperature (37°C). It appears that an almost exponential response in the dependence of plasticiser migration on temperature occurs[1]. To this end experiments were also carried out at 65°C, as the increase in leaching observed at the higher temperature provides for a worse case scenario and identifies those samples leaching phthalate not apparent at the lower temperature. The extraction time chosen was 30 minutes, based on observational studies of children's mouthing behaviour[2].

Standardisation of the units in which leaching rates are reported has not yet taken place. Amongst the units in use include μg/hour/10.3cm^2 (US CPSC)[3], μg/hour/cm^2, μg/g/20 hours (RIVM, Netherlands)[2] and μg/10cm^2/min (LGC, UK)[1,4]. Such variations in units makes direct comparisons of results difficult and efforts are being made to agree on a standard unit. For the purpose of the results to follow the leaching rate used will be μg/10cm^2/min as this appears to be the unit favoured by the EU.

Two methods are described for assessing the leaching rate of DINP from plastic articles. The first method is based on solvent extraction while the second method is based on SPME. Although initially designed to determine phthalate leaching rates they may equally be applicable to measuring organic chemical release from such objects generally.

4.2 EXPERIMENTAL

4.2.1 Reagents and Materials

85μm polyacrylate fibres were purchased from Supelco, Bellifonte, USA.

The water used throughout for preparation of standards was purified by passage through a carbon filter attached to a water cooler. This water, in addition to HPLC water and water from a Millipore system, was analysed by purge and trap – gas chromatography – mass spectrometry and was found to contain fewer traces of organic compounds than the other two sources.

DINP was purchased from Aldrich Chemical Company (Milwaukee, USA) as mixture of C_9 isomers that had an ester content of 99+ %. This was the best grade commercially available. Diallyl phthalate was used as the internal standard and was obtained from Fluka Chemika. Di-(2-ethylhexyl)-phthalate (DEHP) and di-iso-decyl phthalate (DIDP) of 99% purity were received from LGC, UK.

A number of saliva simulants have been reported within the literature[1,2,3,4]. These simulants are typically aqueous solutions of inorganic salts with certain organic constituents added to more closely approximate real saliva. For these studies a simulant formulated according to BS6684:87 was initially chosen due to its widespread use. It consists of both inorganic salts and relevant organic components but does not contain proteinaceous components as such components can provide difficulties at the solvent extraction stage due to the formation of emulsions. BS6684:87 is composed of sodium chloride (4.5g), potassium chloride (0.3g), sodium sulphate (0.3g), ammonium chloride (0.4g), urea (0.2g) and lactic acid (3.0g) dissolved in distilled water and the solution made up to 1000ml. The pH of the solution is adjusted to pH 4.5-5 by the addition drop wise of 5M NaOH.

The E.U.'s Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE) then recommended that a new saliva simulant (suggested by RIVM, The Netherlands) be used as it was observed that micro-organisms were rapidly growing in the original saliva simulant (BS6684:87). The Dutch formulation is composed of

0.82mmol/l magnesium chloride (0.08g/l), 1.0mmol/l calcium chloride (0.11g/l), 3.3mmol/l di-potassium hydrogen phosphate (0.57g/l), 3.8mmol/l potassium carbonate (0.52g/l), 10mmol/l potassium chloride (0.75g/l) and 5.6mmol/l sodium chloride (0.33g/l). The potassium and sodium salts are dissolved in 900mls distilled water. The magnesium and calcium salts are then added. The solution is made up to 1000mls. The pH of the solution is adjusted to pH 6.8 by the addition dropwise of 3M hydrochloric acid (HCl)[2].

Dicloromethane, hexane and methanol were analytical reagent grade and were obtained from LabScan., Dublin, Ireland.

4.2.2 Apparatus

(a) Manual SPME was carried out using an 85μm polyacrylate fibre attached to a Supelco SPME holder. For automated SPME the 85μm polyacrylate fibre was fitted to a Varian 8200 autosampler.

(b) Gas chromatography – mass spectrometry was carried out using a Varian Star 3400 CX gas chromatograph interfaced to a Varian Saturn II ion-trap mass spectrometer. The gas chromatograph was fitted with a 30-metre × 0.25 mm internal diameter, 0.25μm phase loading, J+W DB-5 capillary column.

(c) Concentration of solvent from the solvent extractions was carried out using a Zymak TurboVap concentrator. This instrument consists of a heated water bath into which large glass thimbles containing the solvent to concentrated is placed. Nitrogen gas is blown over the top of the thimbles and vented.

4.2.3 Glassware preparation

Due to the ubiquitous nature of phthalates a strict cleaning regime had to be adopted to ensure that there was no contamination of laboratory glassware. All glassware was thoroughly cleaned using detergent and water followed by rinsing with hexane. Any residual hexane was allowed evaporate from the glassware before use. The cleaning regime was validated as blank analyses did not highlight contamination.

4.2.4 Simulation conditions

Plastic discs of approximately $10cm^2$ (or where the size of the product was small a $5cm^2$ test piece) were punched from the sample using a metal wad-punch, also known as a leather punch. The discs were washed with HPLC grade water to remove any small loose fragments of plastic and then left to stand overnight before proceeding with the analysis. Small pieces of plastic in the saliva simulant resulted in artificially high phthalate ester migration rates being obtained as these fragments were effectively exhaustively extracted of plasticiser at the later solvent extraction stage. The disc was placed in a 250ml conical flask, with 10 glass balls and 50mls of saliva simulant. The flask is glass stoppered and shaken at a pre-determined temperature (37° or 65°C) using a Gallenkamp flask shaker for 30 minutes. The glass balls simulate the slight abrasive effects experienced by the toy in the child's mouth. For experiments carried out @ 65°C metal balls were used. These are heavier than the glass type and therefore contribute more to leaching due to greater abrasive effect.

4.2.5 Liquid-liquid extraction

After 30 minutes shaking, the sample was removed from the conical flask and the saliva simulant transferred to a separating funnel. The flask was rinsed with a 25ml portion of the extracting organic solvent, dichloromethane, and the rinsings transferred to the separating funnel. One liquid-liquid extraction was carried out with this portion and repeated twice with fresh 25ml portions of dichloromethane. The resulting 75ml organic extract was concentrated to near dryness by evaporation of the dichloromethane using a Zymark turbo-vap® concentrator and then reconstituted in 1ml hexane. Analysis of this concentrate by GC-MS and subsequent calculations resulted in the leaching rate of the phthalate ester from the original sample being obtained.

4.2.6 Solid Phase Microextraction

SPME analysis of the saliva simulant for leached phthalates was carried out using the 85μm polyacrylate fibre, as this fibre is not subject to the same degree of phthalate

carryover as the more commonly used PDMS fibre. 1.2mls of sample/standard was placed in a 2ml vial and extracted in the immersion mode due to the relatively low volatility of the analyte in question. A thirty minute extraction time with agitation was found to be sufficient and, due to the GC run time being greater than this, it did not affect overall analysis times. Desorption of the fibre at 320°C for 5 minutes with splitless injection for 0.7 minutes ensured that there was minimal analyte carryover. This can be problematic at lower desorption temperatures. The use of a low volume injector insert was found to improve overall chromatography[5,6] by reducing band broadening.

4.2.7 Gas chromatography-mass spectrometry parameters

The GC was held at 40°C for 4mins, programmed at 10°C/min to 300°C, and held isothermally at 300°C for 5 minutes. Helium was used as the carrier gas at a flow rate of 1ml/min. The GC-MS transfer line was held at 210°C and data was acquired in full scan mode, mass range monitored was 35 – 300amu. Electron Impact was the ionisation mode and the scan rate was 0.7seconds/scan.

Figure 4.1 summarises the method for the determination of phthalate ester leaching from plastic items

4.3 RESULTS AND DISCUSSION

DINP eluted as a series of unresolved peaks between 30.5 and 33.0 minutes (**Figure 4.2**). Mass-to-charge ion 149 was found to be DINP's base peak so the abundance of this ion between 30.5 and 33.0 minutes formed the basis of the quantitation procedure. Another quantitation approach which is commonly used is obtaining the ratio of the relative peak heights of two of the isomers in the mixture. As DINP from a number of sources was being investigated in this study this approach was not adopted.

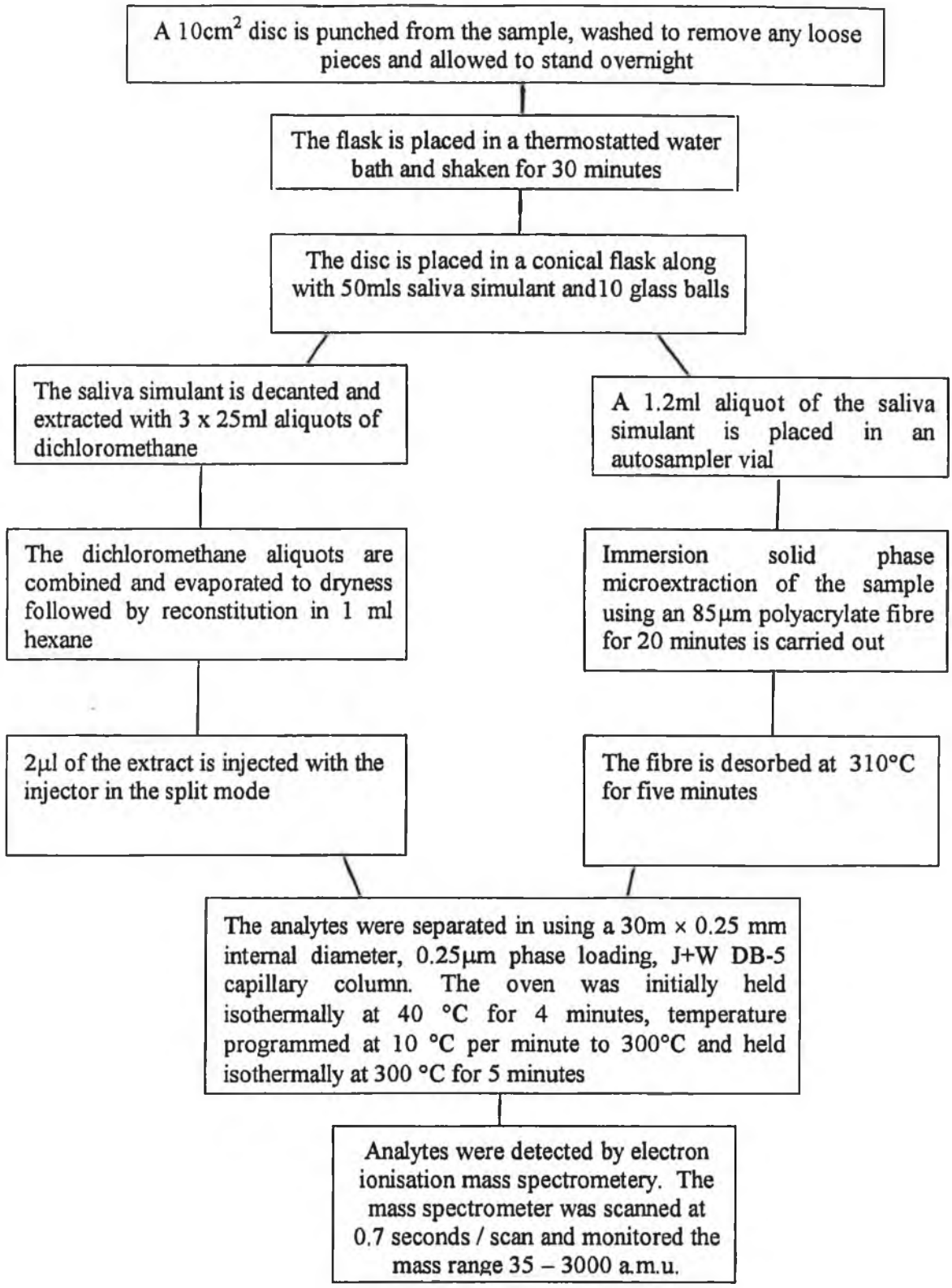

Figure 2.1: Summary of method for the determination of phthalate leaching rates from plastic items

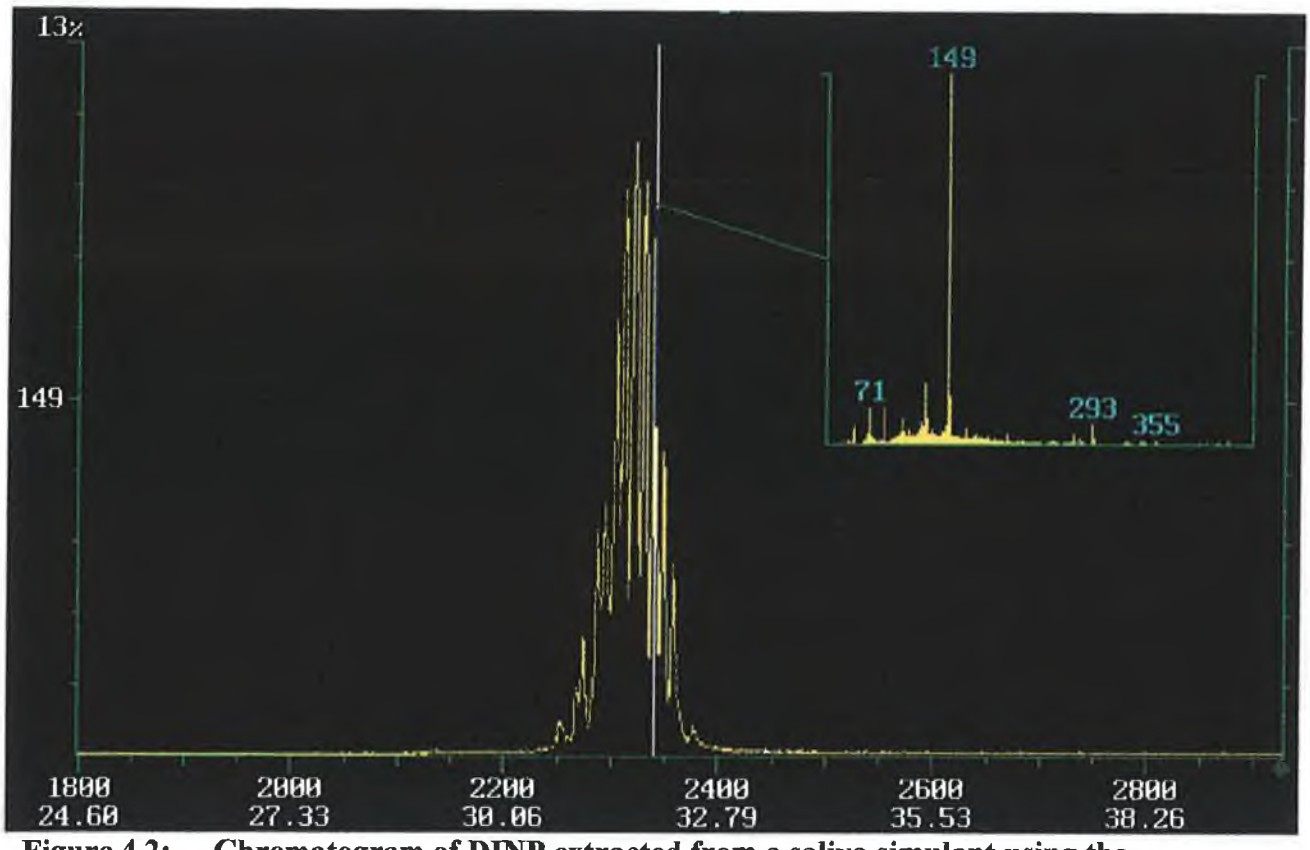

Figure 4.2: Chromatogram of DINP extracted from a saliva simulant using the Polyacrylate Fibre (mass spectrum inset)

4.3.1 Solvent Extraction

(i) *Linear Range*: Standards were prepared gravimetrically in the range 5mg/L to 500mg/L. 2µl injections were carried out with good linearity being observed in this range. Calibration curve correlation coefficents were found to be between 0.995 and 1.000.

(ii) *Repeatability:* Repeatability of the injection was assessed by carrying out five injections of a 10mg/L DINP standard. The percentage coefficent of variance was calculated as 5.8%. To determine repeatability of the solvent extraction step 50mls of saliva simulant was spiked with DINP at a concentration of 4 mgs/l. For three replicate extractions RSD was calculated as 13.5%.

(iiii) *Recovery Data:* Recoveries were calculated by preparing a DINP standard in propan-2-ol, spiking three conical flasks containing 50mls saliva

simulant and treating the spiked flask as a sample. Average recovery was calculated as 76% (RSD=13.5%)

(iv) *Limit of Detection*: To determine the limit of detection 50mls of saliva simulant was spiked with decreasing amounts of DINP followed by subsequent extraction. The limit of detection was calculated as that level which consistently gave a signal to noise ratio of 5 which in this case was 30μg/50mls. Assuming a $10cm^2$ sample disc shaken for 30 minutes in 50 mls of saliva yielded a concentration of 30μg/50mls this would equate to a leaching rate of $1\mu g/10cm^2$/min.

4.3.2 Solid Phase Microextraction

The fibre of choice for extraction of DINP is the 85μm polyacrylate fibre. It effectively and selectively extracts the analyte of interest at the levels required. While initial investigations showed the 100μm and 7μm fibre coatings to be also capable of this, these coatings were not chosen due to the coating strongly retaining the analyte resulting in subsequent carryover. Due to the semi-volatile nature of the analyte immersion sampling with agitation was chosen.

(i) *Determination of extraction time:* A 10mg/L standard was prepared and fresh aliquots of the standard extracted for times varying from 5 minutes to 120 minutes. Examination of **Figure 4.3** shows that equilibration has not been reached after two hours extraction, as can be seen by the continually rising increase in peak areas with increasing extraction time. Agitation of the fibre in the sample is by means of vibration using a small electric motor attached to the autosampler. The true equilibration time was not determined as after two hours of fibre vibration friction of the SPME needle against the septum vial started the septum to slightly burn. An extraction time of 30 minutes was decided upon as this is less than the GC run time and therefore would not unnecessarily increase analysis time. It also achieves the necessary method sensitivity.

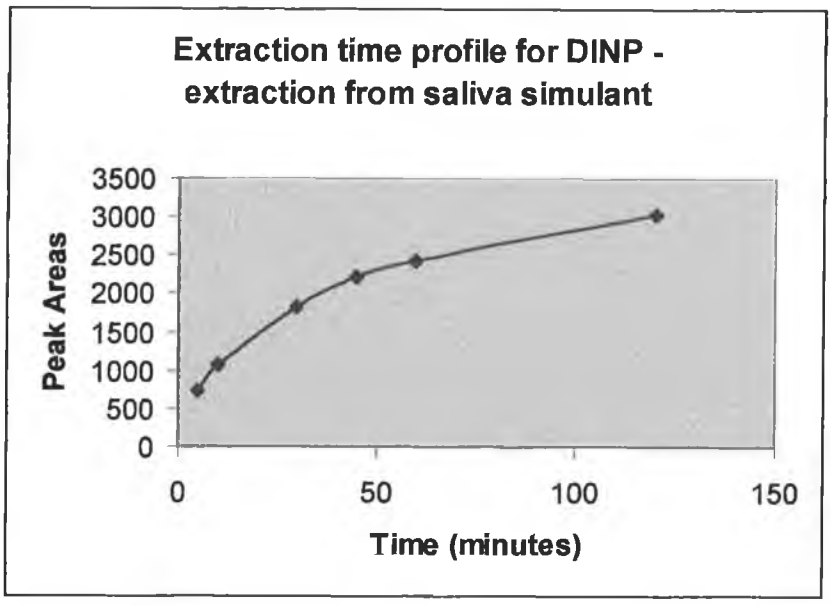

Figure 4.3 Graph showing variation in amount extracted with time

(ii) *Optimising desorption conditions:* The maximum operating temperature of the 85μm polyacrylate fibre is 320°C. Ideally a temperature lower than the maximum operating temperature should be used to maximise the fibre life time. For this application it was necessary to use the maximum temperature as lower temperatures resulted in analyte carryover between runs occurring.

(iii) *Repeatability of SPME analysis:* Standards were prepared at three different concentration levels and extracted. 0.6mg/L, 1mg/L and 10mg/L standards were extracted five times and RSD calculated as 8%, 14% and 22%, respectively. Such repeatability suggests that SPME may be better suited as a screening method for DINP leaching than the basis for an analytical method. It is thought that this poor repeatability is related to the low solubility of DINP in aqueous matrices. DINP is soluble in water up to 1mg/L after which the solution turns cloudy.

(iv) Linear Range: Extraction of DINP from aqueous matrices is linear in the range 0 – 1.1 mg/L. For concentrations greater than 1.1mg/l a sudden large increase in peak area is observed. A proportional increase in peak area is again observed with further increases in concentration up to 25mg/l. The two linear ranges observed are most likely solubility related with the higher range being possibly due to

DINP precipitating out of solution followed by a physical trapping of the precipitate on the fibre.

(v) SPME Method Detection Limit: The limit of detection was calculated as 0.3mg/l. A standard was prepared at this concentration and analysed 10 times (RSD = 8.6%). Peak areas greater than five times the signal to noise ratio of the baseline were observed. Assuming a $10cm^2$ sample disc shaken for 30 minutes in 50 mls of saliva yielded a concentration of 0.3mg/l this would equate to a leaching rate of $0.5\mu g/10cm^2/min$.

4.3.3 Sample screening

A sample of eighteen commercially available samples of children's toys and childcare articles, considered to be representative of the general market, were initially screened to identify the phthalates present. These samples consisted of teething rings, children's soothers, cups, plates, armbands and a variety of different toys. Screening was carried out by shaking approximately 0.05g of each sample in 5mls hexane. The organic extract was subsequently analysed by direct injection of a 0.5µl aliquot onto a gas chromatograph with a 100:1 injector split. Detection was by ion-trap mass spectrometer. Instrument parameters are as outlined in **Section 4.2.7.** Results are summarised in **Table 4.1.**

Table 4.1 Identification of phthalate esters present in 18 commercial samples.

Phthalate	Samples Phthalate Present in
DINP	28% or 5 of 18
DEHP	6% or 1 of 18
DIDP	0
No DINP, DEHP or DIDP	72% or 13 of 18

The predominant phthalate was found to be DINP which is in common with surveys carried out by the US Consumer Product Safety Commission[3], Green peace[7] and LGC UK[4]. The majority of toys tested were not found to contain any of the three phthalates tested for. Some samples were quite hard in nature and may not be

plasticised to any significant degree. The soft nature of other samples suggested that they most probably were plasticised, the plasticiser used being perhaps an adipate or sebecate or one of the phthalates not tested for such as dibutyl phthalate or butyl benzyl phthalate. The amount of phthalate contained in each sample was not calculated as both the CSPC and LGC have shown that the amount leached is independent of the amount contained in the sample originally[3,4]. Although this screening exercise is not a comprehensive review of the distribution of use of phthalates in PVC toys available on the Irish market it does support the opinion that DINP is the most common phthalate used.

4.3.4 Determination of leaching rates from commercially available samples

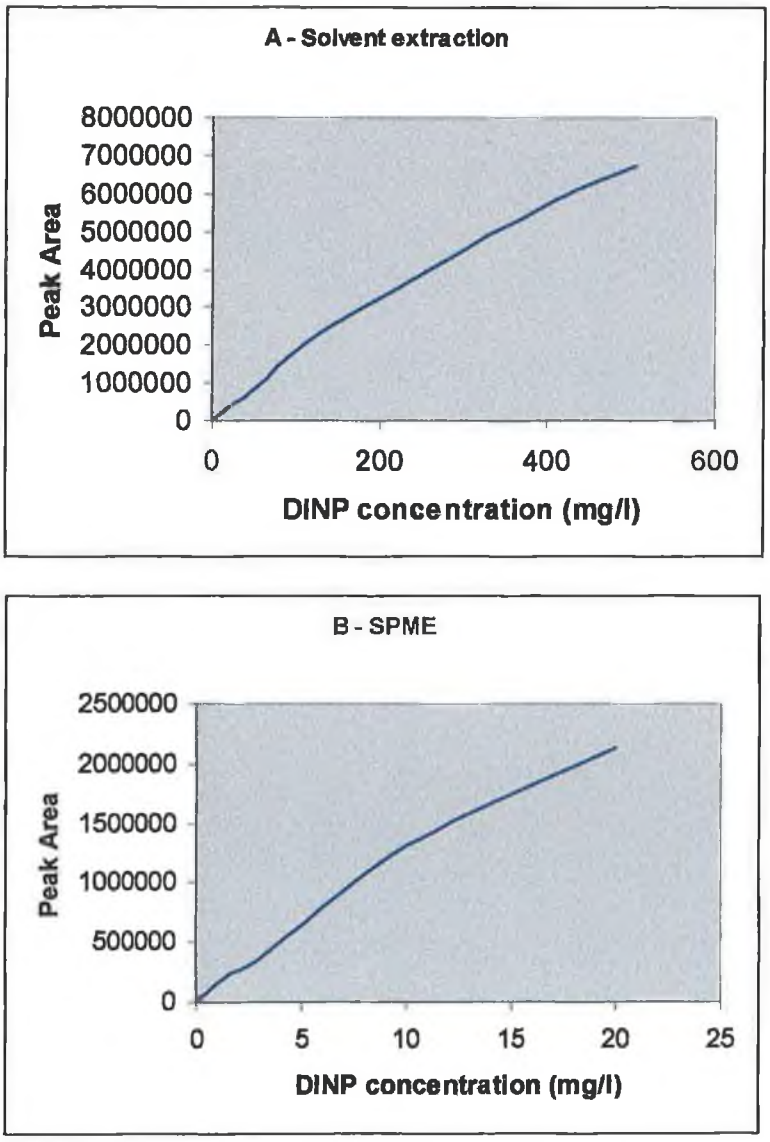

Figure 4.4 Calibration curves for DINP (a) solvent extraction and (b)SPME

Those samples known to contain DINP were then subjected to phthalate leaching as outlined in **Section 4.2.4**, using the saliva simulant suggested by RIVM, The Netherlands. Two sets of samples underwent leaching. One set of samples was analysed by solvent extraction-gas chromatography-mass spectrometry using the procedure described in **Section 4.2.5**. The other set was analysed by SPME-gas chromatography-mass spectrometry in accordance with **Section 4.2.6**

Calibration standards were prepared gravimetrically. Calibration curves are shown in **Figure 4.3.** All samples were shown to leach Diisononyl phthalate as can be seen from the results shown in **Table 4.2.** Only sample three was found to leach DINP above CSTEE's guideline value. This value is 1.2mg/10cm^2/6 hours which assuming a proportional amount is extracted in one minute is 3.3ug/10cm^2/min. Migration rates calculated using both methods agreed with each other to varying degrees, from within 6% (sample one) to a maximum of 58% (sample two). The reason for the difference in results between the two methods, in particular for sample two, require further investigation. As a limited number of repeat measurements were made on each sample a statistical evaluation of the significance of the differences was not attempted.

One possible reason for the variations may be related to the solubility of DINP. It was observed that the saliva simulant solution, which is completely clear at the start, turns turbid after an agitation period. Analysis of these solutions were shown to contain amounts of DINP above what would be expected to dissolve in an aqueous environment. RIVM noted that after filtering through a disposable 0.45μm filter the resulting clear solution does not contain DINP above 0.1μg/L^2. This could, they reasoned, be caused by the presence of PVC particles or by the over-saturation of the

solution. Saliva simulant was spiked with DINP until the solution turned turbid, which occurred at a concentration of 35ug/ml. The spiked simulant was filtered and again the resulting clear solution did not contain DINP above 0.1μg/L. From this they concluded that simulant can be over-saturated and release of plasticiser may not depend on solubility properties but mainly on mechanical forces to make 'suspension like' solutions.

Table 4.2: Leaching rates measured for commercially available toy samples measured @ 65°C

Sample	DINP Leaching Rate (μg/10cm^2/min)	
	LLE	SPME
1	2.60	2.45
2	0.97	1.53
3	6.70	5.67
4	0.48	0.45
5	1.32	1.60

4.3.5 Determination of leaching rates from reference disks

There are many parameters which influence the final result when calculating the amount of phthalate leaching from a PVC product. Such parameters all contribute to the overall repeatability of the assay. Analytical detection method, calibration and leaching temperature are amongst the more easily controlled contributors to repeatability. Obtaining a representative sample on which to carry out the analysis can be more difficult. The production of plasticised PVC sheets is such that the finished product may not be uniformly plasticised. Further moulding of the sheet into plastic toys and childcare articles produces a finished product from which it can be difficult to obtain two discs of equal thickness. Although problematic this parameter can more easily be assessed than phthalate distribution.

In order to negate the contribution from non-uniform samples in assessing the method, PVC reference disks were obtained from the Laboratory of the Government Chemist, UK. These disks were cut from a commercially produced PVC sheet of defined composition which had a DINP content of 38.5%. LGC previously determined the total plasticiser content of six of these discs by Soxhlet extraction and

gravimetric analysis and confirmed by GC-MS. The mean value, standard deviation and coefficient of variation were calculated to ascertain homogeneity.

Discs were placed in conical flasks with saliva simulant and glass balls and allowed to leach under the conditions outlined in **Section 4.3.2**. The saliva simulant used was the Dutch formulation. Results are shown in **Table 4.3**.

Table 4.3: Leaching rates measured for LGC reference disks @ 65°C

DISC	DINP Leaching Rate(μg/10cm^2/min)	
	LLE	SPME
1	4.0	3.2
2	3.5	2.0
3	3.6	2.4
4	1.6	3.9
Average	**3.2**	**2.9**
Standard Deviation	**1.1**	**0.8**
RSD (%)	**33.7**	**29.7**

Repeatability of DINP leaching rates from reference disks is quite poor. During agitation of the discs in the saliva simulant some discs can be seen to move around in some of the flasks more than others. Transfer of plasticiser from sample to simulant occurs in two distinct phases. The first phase is where plasticiser transfer is entirely controlled by internal diffusion in the PVC itself and the second phase is where transfer is controlled by boundary layer phenomena. For the second phase stirring and composition of the contacting solution is very important. If disc A is freely moving around in conical flask A it will leach more plasticiser than disc B which is stuck to the wall of flask B as it is in contact with more saliva simulant than disc B.

4.3.6 Interlaboratory trial on phthalate ester migration from PVC toys.

As part of a European wide effort to develop a standardised method for the determination of the migration rates of phthalate esters from children's toys an interlaboratory comparison study was organised by the Laboratory of the Government Chemist, U.K[8]. Testing was carried out in accordance with the protocol provided by LGC and results submitted. Six other laboratories from throughout Europe also took

part. The exercise aimed to validate two methods. The first "simulated" method, aimed at representing the human oral environment as far as is practical in the laboratory and was shown to compare closely with the mean *in vivo* migration level of DINP from standard PVC discs as determined by adult human volunteer study. The second "stringent" method, a minor modification of the "simulated" method in terms of mode of agitation and temperature, was shown to compare closely with the maximum migration level of DINP from standard PVC discs as determined by adult human volunteer study. The "simulated" method involved six separate analyses of a reference disk at 37°C using glass balls while the "stringent" method was carried out at 65°C using stainless steel balls for abrasion. Three toy samples and one childcare article were also supplied for testing at 65°C. The protocol used can be found in **Appendix One.** The results obtained for this operator are outlined in **Tables 4.4 to 4.8.**

Table 4.4
Identification of phthalates present in samples presented

Sample Name	Plasticiser present
Reference Disc	DINP
LGC 1	DINP
LGC 2	DINP
LGC 3	DEHP
LGC 4	DIDP

These results are in agreement with other members of the trial and the trial organisers. All participants correctly identified the phthalates present in samples and standards.

Table 4.5
Phthalate migration rates measured from PVC reference disks at 65°C

Sample ID	Phthalate Ester Migration (μg/10cm^2/min)		
	DEHP	DINP	DIDP
LGC Ref A	<0.013	2.74	<1.00
LGC Ref B	<0.013	4.23	<1.00
LGC Ref C	<0.013	2.39	<1.00
LGC Ref D	<0.013	2.43	<1.00
LGC Ref E	<0.013	1.09	<1.00
LGC Ref F	<0.013	4.91	<1.00
Blank	<0.013	<1.00	<1.00

Each participating laboratory was requested to carry out phthalate migration measurements on reference discs at either 37°C or 65°C - for this laboratory 65°C was the temperature assigned. As expected no leaching of DEHP or DIDP was observed as the reference discs did not contain these plasticisers. Leaching of DINP varied from 1.09 to 4.91μg/10cm^2/min for 6 replicate reference discs. Repeatability of the GC-MS analytical detection was calculated as 3.9% for a 250μg/ml standard whereas the corresponding repeatability for the migration rates measured is 46.7%. These results suggest that the agitation / extraction step is the main cause of the variability and were similar to results obtained by the other laboratories. **Table 4.6** shows repeatability and reproducibility for all laboratories. Results obtained at 37°C by other participants are also included.

Table 4.6
Inter-laboratory migration trial results for reference discs

	Reference Disks	
	DINP @ 37 °C	**DINP @ 65 °C**
Mean - All Laboratories	1.43 (μg/10cm^2/min)	8.83 (μg/10cm^2/min)
Repeatability (r) - All Labs	0.89	5.68
CV % (r) - All Labs	21.9	22.7
Reproducibility (R) - All Labs	1.43	7.77
CV % (R) - All Labs	35.3	31.1

Table 4.7
Phthalate migration rates measured from commercial samples at 37°C

Sample ID	**Phthalate Ester Migration (μg/10cm^2/min)**		
	DEHP	**DINP**	**DIDP**
LGC1	<0.013	<1.00	<1.00
LGC2	<0.013	<1.00	<1.00
LGC3	<0.013	<1.00	<1.00
LGC4	<0.013	<1.00	<1.00
Blank	<0.013	<1.00	<1.00

Table 4.8
Phthalate migration rates measured from commercial samples at 65°C

Sample ID	Phthalate Ester Migration (μg/10cm²/min)		
	DEHP	DINP	DIDP
LGC1	<0.013	3.71	<1.00
LGC2	<0.013	1.61	<1.00
LGC3	1.41	<1.00	<1.00
LGC4	<0.013	<1.00	2.86
Blank	<0.013	<1.00	<1.00

Table 4.9 - Inter-laboratory migration trial results for commercial samples leached at 37 °C

Sample	Phthalate Leached	Average Leaching Rate (μg/10cm²/min)	SD	% CV
LGC 1	DINP	0.7	0.25	37.4
LGC 2	DINP	1.2	0.79	64.2
LGC 3	DEHP	1.2	0.06	5.5
LGC 4	DIDP	1.6	0.65	39.43

Table 4.10 - Inter-laboratory migration trial results for commercial samples leached at 65 °C

Sample	Phthalate Leached	Average Leaching Rate (μg/10cm²/min)	SD	% CV
LGC 1	DINP	5.0	1.17	23.3
LGC 2	DINP	3.9	1.29	33.1
LGC 3	DEHP	3.1	1.48	48.5
LGC 4	DIDP	4.1	1.03	25.21

Each laboratory was requested to determine leaching of phthalate from the samples provided at both 37°C and 65°C. Although many laboratories were able to detect phthalate leaching at 37°C (**Table 4.9**) the results shown in **Tables 4.7 and 4.8** show only migration at 65°C was detected by this operator. It is worth noting that these migration rates measured at 65°C are lower than average phthalate migration rates. This can most likely be explained by comparing phthalate recoveries from the single operator (**Table 4.11**) with average phthalate recoveries from all participants (**Tables**

4.12 to 4.14). These recoveries are generally lower than average recoveries - up to 23% for DINP at 65°C.

Table 4.11
Phthalate ester recovery data

Phthalate Ester	Batch	% Recovery
DINP	65°C / Stainless Steel Balls	67
DEHP	37°C / Glass Balls	88
DINP	37°C / Glass Balls	73
DIDP	37°C / Glass Balls	96
DEHP	65°C / Stainless Steel Balls	99
DINP	65°C / Stainless Steel Balls	87
DIDP	65°C / Stainless Steel Balls	76

Table 4.12 - DINP recovery for agitation extraction procedure

	DINP standard (200 µg/ml)	
	% Recovery @ 37 °C	% Recovery @ 65 °C
Mean - All Laboratories	90.02	89.92
SD	8.37	12.22
CV %	9.3	13.6

Table 4.13 - DIDP recovery for agitation extraction procedure

	DIDP standard (200 µg/ml)	
	% Recovery @ 37 °C	% Recovery @ 65 °C
Mean - All Laboratories	87.14	87.97
SD	14.57	11.84
CV %	16.7	13.5

Table 4.14 - DEHP recovery for agitation extraction procedure

	DEHP standard (20 µg/ml)	
	% Recovery @ 37 °C	% Recovery @ 65 °C
Mean - All Laboratories	86.43	87.75
SD	5.87	11.67
CV %	6.8	13.3

Statistical analysis of these results and results from the other participants were carried out by the LGC to LGC-1990 "Guidelines for the Development of Methods by Collaborative Study" which are in accordance with IUPAC - 1987 "[Harmonised] protocol for the Design, Conduct and Interpretation of Collaborative Studies" and ISO 5725-1994 "Accuracy (Trueness and Precision) of Measurement Methods and Results".

The LGC in their report acknowledge that the results represent "a relatively limited and shortened exercise in terms of normal practice for method validation by collaborative trial". The small number of participating laboratories meant that full use of statistical analysis, in terms of accounting for stragglers and outliers, could not be justified. In the majority of cases the results represent laboratories first attempts at the method and this may be partly responsible for the differences observed between individual laboratories. Repeatability and reproducibility would most likely be improved on their repeated use. An additional source of variability may be due to a situation where migration gives phthalate concentrations in the aqueous phase which are higher than the solubility of DINP so that micelles are formed. The metal balls used as a source of mechanical force may be tearing off small fragments from the PVC samples. This may also be contributing to variability and needs to be addressed. Despite the average migration value of all laboratories (1.43 $\mu g/10cm^2/min$) matching closely with that found in the Dutch *in vivo* study (1.38 $\mu g/10cm^2/min$)[2] the variability both within and between labs is large making it difficult to use the method for legal purposes.

A similar attempt by the Dutch to validate their "head over heels" method also produced large variations in results[9]. The method was based on the mechanical agitation of a test specimen in a saliva simulant using their "head over heels" rotator. After agitation the saliva was extracted with iso-octane and the amount of DINP was determined by normal phase HPLC using UV detection at 225 nm. Five laboratories participated. Repeatability for the HPLC and agitation methods was found to be 0.3-8.9% and 2.5-26.7%, respectively. Reproducibility for HPLC and agitation methods was 23.2 and 27.5%, respectively.

4.4 CONCLUSION

Measuring leaching rates of phthalate esters from plastic toys and childcare articles is a two step process. In the first step, the plastic toy is placed in a saliva simulant and the phthalate esters are allowed to leach into the simulant. The second step involves measuring the concentration of the phthalate ester in the saliva simulant.

Different laboratories approach the first step in different ways, the method chosen affecting the amount of phthalate leached. Phthalate leaching was carried out by shaking a $10cm^2$ sample disc in 50 mls of saliva simulant in a thermostatted bath for 30 minutes. This approach was chosen due to its relative simplicity when compared to some of the other methods that require custom built pieces of apparatus.

The techniques used to measure the concentration of phthalate esters in the saliva simulant are similar between laboratories. It generally involves a liquid-liquid extraction of the simulant followed by analysis, in the majority of cases, by GC, but in some cases by HPLC. A method is outlined to replace the lengthy liquid-liquid extraction. It utilises an 85μm polyacrylate SPME fibre immersed in a 1.2ml aliquot of saliva simulant for 20 minutes followed by thermal desorption in the GC's injector at 310°C for 5 minutes.

While SPME outperforms liquid liquid extraction with respect to overall analysis times and ease of use it does suffer from poor repeatability. Due to DINP's sparing solubility in aqueous media SPME may not be a suitable method for the quantitative determination of DINP leaching rates but instead may be better incorporated as part of a rapid screening method. More work is required to ascertain the source of poor repeatability.

Currently laboratories involved in the determination of phthalate leaching rates in the European Union are working to develop and validate a standard method. This method could be used to identify those articles leaching phthalate above a target value, thereby stopping an outright ban on the use of these esters, which in turn, would prevent the introduction of alternative compounds whose health effects have not been

fully assessed. These methods are trying to match the migration rates obtained from *in vivo* studies.

4.5 References

[1] I.P. Axford, A.O. Earls, R.P. Scott and J.H. Braybrook, "Laboratory-Based Agitation Methods for the Determination of Phthalate Plasticiser Migration from PVC Toys and Childcare Articles - 2", LGC Technical Report LGC/199/DTI/002 (1999). Published by LGC, Queen's Road, Teddington, Middlesex TW11 OLY, UK.

[2] National Institute of Public Health, The Netherlands, "Phthalate release from soft PVC baby toys" RIVM Report No. 613320 002 (September 1998). Published by RIVM, PO Box 1, 3720, Bilthoven, The Netherlands.

[3] U.S. Consumer Product Safety Commission, "The Risk of Chronic Toxicity Assocaited with Exposure to Diisononyl Phthalate in Children's Products - Executive Summary", 1998. Published by U.S. Consumer Product Safety Commission, 4330 East West Highway, Bethesda, MD 20814, United States.

[4] A.O. Earls, C.A. Clay, I.P. Axford, R.P. Scott and J.H. Braybrook, "Laboratory-Based Agitation Methods for the Determination of Phthalate Plasticiser Migration from PVC Toys and Childcare Articles", LGC Technical Report LGC/1998/DTI/009 (1998). Published by LGC, Queen's Road, Teddington, Middlesex TW11 OLY, UK.

[5] R. Shirey, High Resolut. Chromatogr., **18** (1995) 495.

[6] T. Gorecki and J. Pawliszyn, Anal. Chem., **67** (1995) 3265.

[7] "Determination of the composition and quantities of phthalate ester additives in PVC children's toys", www.greenpeace.org/~comms/97/pvctoys/other/summary.html.

[8] I.P. Axford, A.O. Earls, R.P. Scott and J.H. Braybrook, "Interlaboratory Validation of Laboratory-Based Agitation Methods for the Determination of Phthalate Plasticiser Migration from PVC Toys and Childcare articles", LGC Technical Report LGC/1999/DTI/004 (1999). Published by LGC, Queen's Road, Teddington, Middlesex TW11 OLY, UK.

[9] TNO Nutrition and Food Research Institute, The Netherlands, "Validation of the method "Determination of Diisononylphthalate in saliva simulant"", TNO report V99.598 (1999). Published by TNO, Ultrechtseweg 48, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

APPENDIX A: Ion abundance criteria for 4-bromofluorobenzene

Mass (M/z)	Relative abundance criteria
50	15 to 40% of mass 95
75	30 to 80% of mass 95
95	Base peak, 100% relative abundance
96	5 to 9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5 to 9% of mass 174
176	>95% but >101% of mass 174
177	5 to 9% of mass 176

APPENDIX B:

Protocol used in the Interlaboratory trial of two methods for the migration of phthalate esters from PVC

Interlaboratory trial of two methods for the migration of phthalate esters from PVC

1. Introduction:

This trial will provide validation data for two methods, developed at LGC, estimating the migration of phthalate esters from soft PVC toys and teethers. The first method carried out at 37°C is representative, as far as possible, of conditions found in the infant oral environment and correlates with mean phthalate plasticiser release values obtained from the Dutch Consensus Group *in vivo* studies. A further method at an elevated temperature (65°C) is designed to provide levels of migration that meet the requirement of the EU Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE), i.e. the maximum phthalate release value obtained from the Dutch Consensus Group *in vivo* studies.

2. Test Methods:

Please see attached as Appendix 1.

3. Workplan:

The study involves the testing of three toy samples, one childcare sample and one specially prepared PVC reference disc. One test should be performed on the toy and childcare samples at each temperature; the PVC reference disc should be tested 6 times at your assigned temperature. An appropriate blank determination and spiked recovery solution should also be run for each batch.

A sufficient number of PVC reference discs have been supplied to enable method familiarity tests to be carried out, if desired.

It is anticipated that, for a single operator, the sample preparation should take no longer than two days to complete and the analytical detection a further one day. A preferred workplan can be found at Appendix 2.

4. Apparatus/reagents supplied:

The following items should have been received:

Quantity	Description		ID No.
10	PVC reference discs	-	LGCREF
1	Thick toy sample (teether)	-	LGC1
1	Section of thin toy sample (inflatable whale)	-	LGC2
1	Section of thin toy sample (swimming armband)	-	LGC3
1	Section of thin childcare sample (high chair covering)	-	LGC4
5	Weighting clips for thin samples		
2g	DINP Standard (Jayflex)		
1g	DIDP Standard (99% pure)		
2g	DEHP Standard (99% pure)		
80	Glass balls		
80	Stainless steel balls		

5. Special instructions for participants:

It is important that any deviation either in analytical conditions, methodology used, or the use of alternative labware, is clearly noted.

There is no need to carry out any statistical analysis as one will performed at LGC once all results have been collated.

Please return the following at the completion of the trial:

- **Result sheets (see Appendix 3)**
- **All cut specimens and used discs.**
- **Copies of all instrument traces**
- **All apparatus/reagents supplied (in the box provided).**

6. Date for completion of trial:

All trial results to be received at LGC by **16 April 1999.** If for any reason this target date is impossible please let us know as soon as possible from receipt of the trial documentation and samples.

Determination of Phthalate Ester Plasticiser Migration from PVC

Introduction

This method describes the identification and measurement of migrated phthalate ester plasticisers from PVC toys and childcare articles.

- The method has been defined to obtain values for phthalate ester migration found in the Dutch Consensus Group *in vivo* studies; i.e. a value of 1.38 µg/10cm^2/min, the mean level of di-isononyl phthalate (DINP) found to have migrated from the Dutch Consensus Group's standard PVC disc during the *in vivo* studies and an extreme value of 9.0 µg/10cm^2/min, the highest level of DINP found to have migrated from an actual teether product during the Dutch Consensus Group's *in vivo* studies.

- Using LGC's PVC reference disc, which has been correlated with the Dutch Consensus Group's standard PVC disc, a mean value of ~1.38µg/cm^2/min DINP migration can consistently be obtained through mechanical pounding of the reference disc with glass balls in artificial saliva using a horizontal shaking water bath maintained at 37°C. Replacement of the glass balls with stainless steel balls and raising the temperature (65°C) has been shown to consistently provide a value of ~9.0 µg/cm^2/min DINP migration from the reference disc.

- The migration values obtained are by direct measurements and do not require a correction factor thus eliminating multiplication uncertainties.

Methodologies

- Qualitative identification of the phthalate ester plasticisers present in the sample.
- Migration of phthalate ester plasticisers using glass balls in artificial saliva at 37°C.
- Migration of phthalate ester plasticisers using stainless steel balls in artificial saliva at 65°C.

1. Equipment

1.1 Cork borer (punch)/Scissors/knife,
1.2 10mL glass test-tube,
1.3 Nitrogen and/or air line,
1.4 250mL wide neck glass conical flask,
1.5 250mL stoppered glass separating funnel,
1.6 Kuderna-Danish (K-D) apparatus with a 150mm vigreux reflux column and 10mL glass tube sufficient to evaporate 150 mL of solvent or an appropriate solvent evaporation system*,
**solid phase extraction systems will be investigated in the future.*
1.7 Steam/Water bath (~100°C)
1.8 Stainless steel balls (Size 12mm diameter, weight ~7g),
1.9 Glass balls (Size 10mm diameter, weight ~1.2g),
1.10 Linear horizontal (transverse) shaking water bath with a shaking amplitude of 38mm and an oscillation speed of 200 strokes per minute,

1.11 Metal tweezers,
1.12 Aluminium foil,
1.13 Conical glass funnel (60 mm top diameter),
1.14 Filter paper (9cm ⌀ Whatman 1 or equivalent).
1.15 1 mL Glass pipette (grade A).
1.16 General volumetric glassware (grade A).
1.17 Metal clip for weighting PVC sheeting.
1.18 GC-MS*
*or GC-FID or reverse phase HPLC.

The following precautions need to be observed to eliminate any possible sources of contamination.

a) *Glassware should be retained for the exclusive use for phthalate studies.*
b) *A strict cleaning regime should be adopted for all apparatus/glassware.* ***Wash all glass, both before and after use, by hand (not washing machine), acid rinse (5% nitric acid), double rinse with distilled water and flush with acetone/methanol (1:1). Dry using an air-line.***
c) *Glassware for qualatitative determinrtion of plasticiser content should be kept separate from migration glassware.*
d) *Only non-PVC labware should be used including stoppers, caps, liners, tubing etc.*

2. Reagents

2.1 **Simulant saliva solution (Dutch formulation):**

(0.82 mmol/L)	Magnesium chloride ($MgCl_2$)	0.08 g/L
(1.0 mmol/L)	Calcium chloride ($CaCl_2$)	0.11 g/L
(3.3 mmol/L)	Di-potassium hydrogen phosphate (K_2HPO_4)	0.57 g/L
(3.8 mmol/L)	Potassium carbonate (K_2CO_3)	0.52 g/L
(5.6 mmol/L)	Sodium chloride (NaCl)	0.33 g/L
(10.0 mmol/L)	Potassium chloride (KCl)	0.75 g/L

Dissolve the potassium & sodium salts in 900 ml of distilled water. Then add the magnesium & calcium salts. Make the solution up to 1000 ml. The pH of the solution is adjusted to pH 6.8 by the addition dropwise of 3 M hydrochloric acid (HCl).

2.2 Dichloromethane (CH_2CL_2) [HPLC grade].
2.3 Sodium sulphate ($NaSO_4$) [anhydrous].
2.4 n-Hexane (C_6H_8) [HPLC grade].
2.5 Propan-2-ol {$(CH_3)_2CHOH$} [HPLC grade].
2.6 Phthalate ester standards:
2.6.1 Di-butyl (DBP)
2.6.2 Butyl Benzyl (BBP)
2.6.3 Di-isodecyl (DIDP)
2.6.4 Di-n-octyl (DNOP)
2.6.5 Di-isononyl (DINP)
2.6.6 Bis-(2-ethylhexyl) (DEHP)

2.7 (2.7.1-6) Prepare a series of stock standard solutions of the individual phthalate esters in n-Hexane (2.4) as shown in Table 1.

Table 1.

	Phthate Ester	Concentration
2.7.3	Di-isodecyl (DIDP)	5000µg/mL
2.7.5	Di-isononyl (DINP)	5000µg/mL
2.7.1	Di-butyl (DBP)	200 µg/mL
2.7.2	Butyl Benzyl (BBP)	200 µg/mL
2.7.4	Di-n-octyl (DNOP)	200 µg/mL
2.7.6	Bis-(2-ethylhexyl) (DEHP)	200 µg/mL

2.8 (2.8.1-2) A series of phthalate ester calibration standards are prepared from the 5,000µg/mL stock standard solution (2.7.3& 2.7.5) at 50, 100, 250, 400 and 500µg/mL concentrations in n-Hexane (2.8).

2.9 (2.9.1-4) A series of phthalate ester calibration standards are prepared from the 200µg/mL stock standard solution (2.7.3& 2.7.5) at 2, 5, 10, 15 and 20µg/mL concentrations in n-Hexane (2.8).

2.10 (2.10.1-6) Recovery phthalate ester solutions. A recovery solution to be prepared using the stock standard solution (2.7.1-6) and prepared in propan-2-ol (2.5) at the following concentrations shown in Table 2:

Table 2.

	Phthate Ester	Concentration
2.10.3	Di-isodecyl (DIDP)	200µg/mL
2.10.5	Di-isononyl (DINP)	200µg/mL
2.10.1	Di-butyl (DBP)	20 µg/mL
2.10.2	Butyl Benzyl (BBP)	20 µg/mL
2.10.4	Di-n-octyl (DNOP)	20 µg/mL
2.10.6	Bis-(2-ethylhexyl) (DEHP)	20 µg/mL

3. Identification of plasticisers in PVC

3.1 Approximately 0.05g of the sample is taken and placed into a stoppered 10 mL glass test-tube (1.2).

3.2 5mL of n-Hexane are added to the test-tube (1.2) stoppered and shaken for about 30 seconds.

3.3 Transfer a portion of the n-Hexane into a capped vial for GC/MS analysis*.

3.4 Compare the obtained GC-MS* spectra to known spectra or phthalate ester standards to allow qualitative identification of phthalate ester plasticisers.

**or alternative technique.*

4. Dynamic Migration of Phthalate Ester Plasticisers from PVC.

4.1 Sample Preparation

4.1.1 Cut a disc of the test material (with minimal cutting) using a cork borer (1.1) of approximately 10 cm^2 surface area (accounting for both sides and edges - where the edge is >1mm thick).

4.1.2 Measure surface area.

4.1.3 Rinse with deionised water to remove any particulates.

4.1.4 Where a disc has an edge is <1mm thick a metal clip (1.17) is attached to weight the sample.

4.2 Migration of Phthalate Ester Plasticisers from PVC using Glass Balls into Artificial Saliva at 37°C.

4.2.1 Preheat, for 20 minutes, 50ml of freshly prepared saliva (2.1) and 10 glass balls (1.8) in a 250 mL wide neck conical flask (1.4) in a shaking water bath (1.9) maintained at 37 ± 2 °C.

4.2.2 Place the 10 cm^2 disc (4.1) into the preheated 50mL of saliva and cover the the 250 mL conical flask with aluminium foil (1.11).

4.2.3 Shake the 250 mL conical flask for 30 minutes at an agitation speed of 200 oscillations per minute and an amplitude of 38 mm.

4.2.4 After 30 minutes carefully decant the saliva solution from the 250 mL conical flask into a stoppered 250 mL separating funnel (1.5) [*use the glass stopper to stop the balls falling into the 250 mL separating funnel*].

4.2.5 Replenish the 250 mL conical flask containing the test material and glass balls with a further 50 mL of fresh pre-heated artificial saliva and shake for 30 minutes at 37° C.

4.2.6 Remove the disc using tweezers (1.10) and add the saliva solution to the same stoppered 250 mL separating funnel used in para **4.2.4**.

4.2.7 Add 25 mL of Dichloromethane (2.2) to the conical flask and gently swirl to absorb any phthalates on the balls or sides of the glass vessel and add to the stoppered 250 mL separating funnel used in para **4.2.4**.

4.2.8 Repeat para **4.2.7**.

4.2.9 Shake, vigorously, the stoppered 250 mL separating funnel for approximately 30 seconds, releasing pressure periodically.

4.2.10 Allow the phases to separate and filter the lower solvent layer into a K-D tube (1.13) via a glass funnel lined with filter paper (1.12) containing 1-2 g sodium sulphate (2.3) [*to trap particles and moisture*].

4.2.11 Add 50 mL of Dichloromethane (2.2) into the stoppered 250 mL separating funnel.

4.2.12 Repeat paras **4.2.9 & 4.2.10.**

4.2.13 Repeat paras **4.2.11 & 4.2.12.**

[*A total volume of 150 mL of Dichloromethane (2.2) will have been used to extract the phthalate esters from the saliva*]

4.2.14 Transfer the K-D tube to a steam bath and evaporate down to approximately 5ml. Remove from the steam bath (1.7) and gently evaporate the remaining solvent to almost dryness using a flow of air or nitrogen.

4.2.15 Add by means of a glass pipette 2.0 mL of n-Hexane (2.8), swirl for 30 sec to re-dissolve the phthalate ester, swirling the n-Hexane to wash any phthalate esters from the sides of the glass tube.

4.2.16 Transfer the solution into a capped vial for GC/MS*, ensuring caps are tightly crimped.
**or alternative technique.*

4.2.17 If necessary the solution may require dilution to meet the limits of the calibration curve.

4.3 Migration of Phthalate Ester Plasticisers from PVC using Stainless Steel Balls in Artificial Saliva at 65°C.

4.3.1 Preheat, for 20 minutes, 50ml of freshly prepared saliva and 10 stainless steel balls (1.8) in a 250 ml wide neck conical flask (1.4) in a shaking water bath (1.9) maintained at 65 ± 2 °C.

4.3.2 Place the 10 cm^2 disc (4.1) into the heated 50ml of saliva and cover the the 250 mL conical flask with aluminium foil (1.11).

4.3.3 Repeat as described in paras 4.2.3-4.2.17.

5. Blank Solution.

5.1 With each batch of samples a 50 mL blank saliva solution [*which has not been in contact with any PVC sample*] should be run as a sample as described in paras 4.2.1-4.2.3 or 4.3.1-4.2.3 *(dependent on temperature and type of ball used)* following the second 30 minute replenishment extraction procedure described in paras 4.2.7-4.2.17.

5.2 Use the blank solution to blank correct the sample result.

6. Recovery Check.

6.1 A 50 mL blank saliva solution is spiked with 1 mL of a phthalate ester solution (2.10.1-6).

6.2 With each batch of samples the spiked 50 mL blank saliva solution (6.1) should be run like a sample as described in paras 4.2.1-4.2.3 or 4.3.1-4.2.3 *(dependent on temperature and type of ball used)* following the second 30 minute replenishement extraction procedure described in paras 4.2.7- 4.2.17.

6.3 Determine recovery.

7. Determination of Phthalate Esters.

The following GCMS conditions for phthalate determination have been found to be suitable:

Model: 5890 GC with a HP5971A MSD with scan range 50amu to 500amu

Column	30m , 0.25mm I.D and 0.15µm film thickness
Carrier gas	Helium
Flow rate	0.8 ml/min
Injector temperature	290°C
Injection volume	2 µl
injection type	splitless
Detector	MSD
Transfer line temperature	280°C
MSD mode	Electron Impact
Temperature programme	40^0C for 4 min
	From 40^0C to 300^0C at 10^0C/min
	Isothermal 4.00 min
	Total run time is 34 mins

Typical quantitation ions for :	**Tgt ion**	**Q1**	**Q2**	**Q3**
Dibutyl phthalate (DBP)	149	223	278	
Butyl benzyl phthalate (BBP)	149	206	238	
Bis-(2-ethylhexyl) phthalate (DEHP)	149	167	279	
Di-n-octyl (DNOP)	149	279	261	
Di-isononyl phthalate (DINP)	149	293	127	167
Di-isodecyl phthalate (DIDP)	149	307	167	141

Equivalent conditions may be employed for phthalate determination.

8. Calculations.

8.1 Plot a calibration graph of the response against the known standard concentrations.

8.2 From the calibration graph determine the response of phthalate ester found in the blank/sample and interpolate the concentration of phthalate ester in µg/mL correcting for any dilutions.

9. Results.

For each run the following should be calculated:

9.1 The blank result (calculated as described in para 8.2), reported as phthalate ester (µg/mL).

9.2 The sample result (calculated as described in para 8.2), reported as phthalate ester (µg/mL).

9.3 The recovery (determined in para **6.3** calculated as described in para **8.2**), reported as phthalate ester (µg/mL).

9.4 The blank corrected sample result (using the blank and sample result calculated in paras **9.1** and **9.2**) to be reported as phthalate ester (µg/mL).

9.5 **Phthalate ester migration rate,** reported as (µg/10cm^2/min).

Calculation:

Migration rate (µg/10cm^2/min) =

$$\frac{\{\text{Blank corrected sample result (µg/mL)} \times 2\text{ (mL)}\} \times \frac{\text{Area of disc (cm}^2\text{)}}{10\text{ (cm}^2\text{)}}}{60\text{ (minutes)}}$$

10. Test Report

The report shall contain the following:

10.1 Name of the laboratory.
10.2 Reporting analyst.
10.3 Date of report.
10.4 Date of analysis.
10.5 Method identification.
10.6 Sample identification.
10.7 Sample, recovery and blank results including limits of detection (see Appendix 3).
10.8 Modification of the method (if applicable).

11. Health & Safety

11.1 All precautions should be taken in ensuring all chemicals are handled safely (*especially when using large volumes of solvents*) and reference should be made to appropriate data handling sheets before commencing analysis.